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Editors: Vibeke Lind, Mari Vold Hansen & Claudia Arndt



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The 9th International Greenhouse Gas & Animal Agriculture Conference, Nairobi, Kenya, GGAA2025

Book of abstracts

Editors: Vibeke Lind¹ Mari Vold Hansen¹ & Claudia Arndt²

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Preface

On behalf of the Organizing Committee, it was a great pleasure to host you in Kenya and GGAA2025. We are honored to have hosted GGAA for the first time in Africa, a continent whose vibrant landscapes, rich agricultural traditions, and dynamic research communities are central to the future of food system transformation and climate solutions. Hosting GGAA2025 in Kenya, home to the United Nations headquarters in Africa, amplified our visibility and ensured that Africa's priorities are represented on the global stage.

This year marked a milestone in the history of GGAA. With over 450 participants from around 50 countries, GGAA2025 reflected our community's truly global reach. Since its first meeting, GGAA has continued to evolve and grow in scope and themes, reflecting advances in science and the changing realities of agriculture and climate. During the opening ceremony, we highlighted challenges and opportunities for mitigating greenhouse gas emissions from ruminant livestock in Latin America, Africa, and Asia. This year we presented nine themes addressed in eight keynote presentations and 17 breakout sessions across livestock, climate, and sustainability. We also succeeded in sponsoring more than 50 researchers, including many students from non-OECD countries, whose voices are essential for shaping the future of our field.

GGAA2025 placed strong emphasis on scaling solutions and addressing regional priorities, such as carbon finance. With the presence of the World Bank and IFAD, alongside our partners and sponsors, we hosted side events that brought greater focus to specific issues vital to Africa and low- and middle-income countries from Asia and Latin America. The combination of cutting-edge research, regional dialogue, and global policy engagement ensured that GGAA2025 was a platform for academic exchange addressing directly real-world problems.

We warmly thank all those who have made the conference possible: our Scientific Committee, ILRI and NIBIO staff, reviewers, moderators, volunteers, sponsors, partners and participants. Your dedication and support have been invaluable.

Sincerely



Dr. Claudia Arndt
Chair of GGAA2025



Dr. Vibeke Lind
Co-chair of GGAA2025

Acknowledgments

We would like to thank the following people for putting together the GGAA2025 conference.

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We would also like to thank the following people for reviewing all submitted abstracts for the GGAA2025 conference.

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Conference program

Sunday								
Start	End	Entrance/ Foyer	Arabuko	Loita	Cherengani	Maai	Mwina	Maranga
09:00	12:00	Registration	Set up exhibition/ sponsors	Set up exhibition/ sponsors	Set up exhibition/ sponsors		Joint ASGGN & ICAR Feed&Gas workshop, Breeding schemes for lowering GHG emissions from ruminants. (Lunch from 11:30am to 12:30pm)	AgriDENZ: Mitigation of GHG emissions in ruminant production pasture systems (Invite only)
9:30	10:00							
10:00	16:00					SF6 workshop		
17:00	19:00				Opening Ceremony			
19:00	21:00			Opening Cocktail				



Monday									
Start	End	Entrance/ Foyer	Arabuko	Loita	Cherengani	Maai	Mwina	Maranga	Makunga
07:45	08:15	Registration							
08:15	09:00			Keynote Theme 1: Effect of forage characteristics on enteric methane emission across ruminant classes. Presenter: Dr. Sanne van Gastelen, the Netherlands					
09:00	09:45			Keynote Theme 5: Assessing the climate footprint of agricultural systems in the tropics: Carbon sequestration and GHG emissions. Presenter: Dr. Mohamad Habibou Assouma, Benin					
09:45	10:45		Coffee & Poster Theme 1: Impact of Animal Feed and Nutrition on GHG Emissions Theme 5: GHG Emissions from Agricultural Soils and Grazing Systems Theme 9: Measurement Techniques for GHG Emissions						Launch of the first comprehensive book on Africa's Animal Genetic Resources [by African Animal Breeding Network (AABNet)]
10:45	12:15			Breakout Theme 1: Dietary supplementation to reduce ruminant GHG emissions - Feed additives 5 oral presentations5 Speed-poster presentations	Breakout Theme 5: Greenhouse Gas Emissions from Grazing Livestock: Role of Feed, Pasture Access, and Soil Carbon 5 oral presentations5 Speed-poster presentations	Presentation of DaIMA – Dairy Intervention for Mitigation and Adaptation			
12:15	14:00		Lunch/Poster						
14:00	15:30			Breakout Theme 1: Dietary supplementation to reduce ruminant GHG emissions - Feed additives 5 oral presentations5 Speed-poster presentations	Breakout Theme 5: Global approaches to mitigating GHG emissions in agricultural soils and grazing systems 5 oral presentations5 Speed-poster presentations	Breakout Theme 9: Measuring livestock emissions – from sensors to satellites 5 oral presentations5 Speed-poster presentations	GMH Showcase Session (14:00 to 14:45)	FAO Global Livestock Environmental Assessment Model (GLEAM): Exchanging experiences and building connections among users	Launch of the first comprehensive book on Africa's Animal Genetic Resources [by African Animal Breeding Network (AABNet)]
15:30	16:15	GLEAM Cocktail	Coffee & Poster Theme 1: Impact of Animal Feed and Nutrition on GHG Emissions Theme 5: GHG Emissions from Agricultural Soils and Grazing Systems Theme 9: Measurement Techniques for GHG Emissions						
16:15	17:00			Keynote Theme 9: Exhaled breath to assess rumen physiology in dairy cows. Presenters: Prof. Mutian Nui, Switzerland Dr. Richard Dewhurst, Scotland					
17:00	18:00			GMH GreenFeed Network Meeting (Open)	EDF/ANH Presentation & Cocktail				
(Below the stairs)			GMH GreenFeed Network Meeting (Open)	EDF/ANH Presentation & Cocktail					
18:00	19:00			GMH GreenFeed Network Meeting (Invite Only)					

Tuesday							
Start	End	Entrance/ Foyer	Arabuko	Loita	Cherengani	Maai	Mwina
07:45	08:15	Registration					
08:15	09:00			Keynote Theme 2: Animal health as a climate solution. Presenters: Prof. Nick Wheelhouse, England Dr. Seyda Özkan-Gülzari, The Netherlands			
09:00	09:45			Keynote Theme 2 and 3: Reducing methane emissions in ruminants: Genomic and metagenomic selection tools Presenters: Dr. Suzanne Rowe, New Zealand			
09:45	10:45		Coffee & Poster Theme 1: Impact of Animal Feed and Nutrition on GHG Emissions Theme 2: Animal Health, Genetics, and Genomics for Climate Resilience Theme 3: Rumen Microbial Genomics and Nutrition Theme 4: Manure Management				
10:45	12:15			Breakout Theme 1: Advancing animal nutrition for low-emissions livestock system 5 oral presentations 5 Speed-poster presentations	Breakout Theme 2: Genetics meets climate action: Breeding strategies for low-emission livestock 5 oral presentations 5 Speed-poster presentations	Breakout Theme 3: Rumen microbiota for sustainable methane mitigation and livestock efficiency 5 oral presentations 5 Speed-poster presentations	
12:15	14:00		Lunch/Poster				
14:00	15:30			Breakout Theme 1: Forage-based strategies for reducing enteric methane emissions 5 oral presentations 5 Speed-poster presentations	Breakout Theme 2: Breeding resilience: Integrating emissions, health and environmental adaptation 5 oral presentations 5 Speed-poster presentations	Breakout Theme 4: Manure management for a changing climate 5 oral presentations 5 Speed-poster presentations	WB Event on Financing and Implementation
15:30	16:15		Coffee & Poster Theme 1: Impact of Animal Feed and Nutrition on GHG Emissions Theme 2: Animal Health, Genetics, and Genomics for Climate Resilience Theme 3: Rumen Microbial Genomics and Nutrition Theme 4: Manure Management				
16:15	17:00				Keynote Theme 4 and 6: Management practices to increase circularity at farm level by reducing nutrient losses from manure. Presenter: Dr. Sonja Leitner, Kenya		
17:00	17:30						
17:30	19:00						
19:00	21:00	DSM Cocktail (invite only) (Below the stairs)		Livestock and Climate Solutions Hub Cocktail Event (Invite only)	Feed and Nutrition Network Meeting		

Wednesday								
Start	End	Entrance/ Foyer	Arabuko	Loita	Cherengani	Maai	Mwina	Maranga
07:45	08:15	Registration						
08:15	09:00	& Maasai Market		Keynote Theme 7: Opportunities for data based on-farm estimation of greenhouse gas emissions from ruminant farming systems. Presenter: Prof. Matti Pastell, Finland				
09:00	09:45			Keynote Theme 8 : Scaling up livestock-based mitigation in countries and improving GHG inventories - The perspective of an international development finance institution. Presenter: Anne Mottet, Italy				
09:45	10:45		Coffee & Poster Theme 1: Impact of Animal Feed and Nutrition on GHG Emissions Theme 6: Circular Economy Theme 7: Scaling and Modeling of GHG Emissions Theme 8: National GHG Inventory and Policy Frameworks with Carbon Finance Mechanisms				From Data to Action: Scaling Climate-Smart Livestock Practices in Pastoral and Agro-Pastoral Systems. Stocktaking: what we know from research and application	
10:45	12:15			Breakout Theme 1: Innovative feeding strategies for reducing methane emissions 5 oral presentations5 Speed-poster presentations	Breakout Theme 6: Innovations in circular livestock systems for climate-smart agriculture 5 oral presentations5 Speed-poster presentations	Breakout Theme 7: Modeling and mitigating enteric methane emissions in ruminants - Perspective on diet, management, and technological interventions 5 oral presentations5 Speed-poster presentations		MIPCLAN- Mitigation practices for low-carbon Livestock in sub-Saharan Africa (Living lab).
12:15	14:00		Lunch/Poster					
14:00	15:30			Breakout Theme 1: Nutritional approaches to minimize GHG emissions 5 oral presentations5 Speed-poster presentations	Breakout Theme 8: From data to action - tackling enteric methane in livestock systems 5 oral presentations5 Speed-poster presentations	Breakout Theme 7: Scaling and modelling of GHG emissions 5 oral presentations5 Speed-poster presentations	From Data to Action: Scaling Climate-Smart Livestock Practices in Pastoral and Agro-Pastoral Systems: Learning by doing: experiences from private sector and development/ conservation programs.	
15:30	16:15		Coffee & Poster Theme 1: Impact of Animal Feed and Nutrition on GHG Emissions Theme 6: Circular Economy Theme 7: Scaling and Modeling of GHG Emissions Theme 8: National GHG Inventory and Policy Frameworks with Carbon Finance Mechanisms					
16:15	17:15				Closing ceremony			
17:15	17:30							
17:30	19:00							
19:00	21:00			Conference dinner				

Day	Conference tours
Thursday 9th Oct:	Tour 1: Maramba Tea Factory, Brown's Cheese Factory and Giraffe Centre
	Tour 3: Kapiti Research Station, KALRO Katumani
	Tour 6: Crescent Island Game Conservancy
	Tour 8: ILRI Nairobi and Kapiti Research Station
	Tour 9: KALRO Naivasha and Dairy Farming
	Tour 10: Kajiado Modernized Pastoral Farms, The Wildlife Foundation Centre and Maasai Nickson Parmisa's Farm
	Tour 11: Nairobi National Park, Sheldrick Elephant Orphanage, Utamaduni Craft Centre and Mamba Village
	Tour 12: Nairobi National Park
	Accompanying person tours
Monday, 6th Oct:	Nairobi National Park, Sheldrick Elephant Orphanage, Utamaduni Craft Centre and Mamba Village
Tuesday, 7th Oct:	Crescent Island Game Conservancy
Wednesday 8th Oct:	Brown's cheese and Maramba Tea

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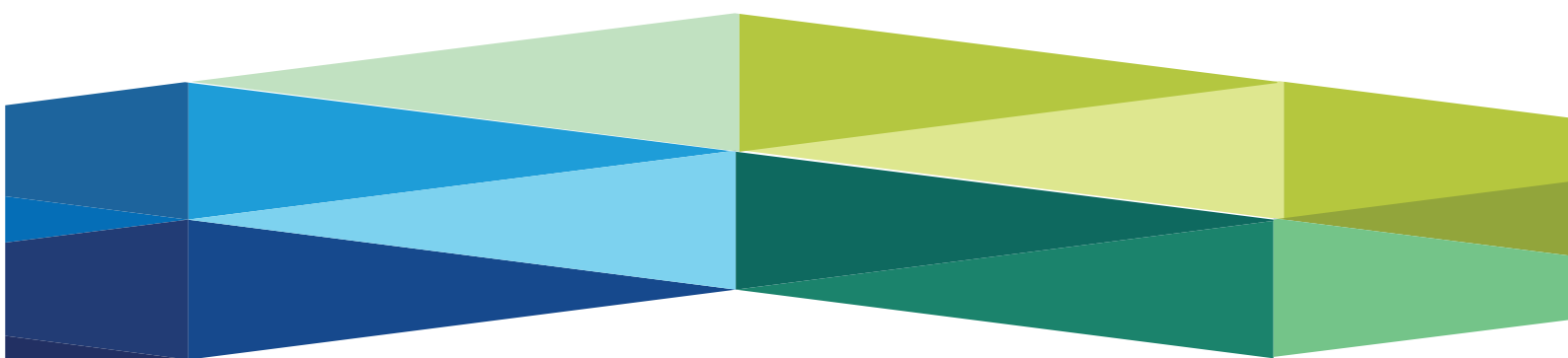
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Keynote abstracts



499 Challenges and opportunities for mitigation of greenhouse gas emissions from ruminant livestock in the context of sustainable production in Latin America

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Abstract

Livestock production plays a vital role in Latin America's economy, food security, social wellbeing and culture, while also providing essential ecosystem services. However, it also contributes significantly to greenhouse gas (GHG) emissions, particularly methane (CH₄) and nitrous oxide (N₂O), from the enteric fermentation and manure management chain. To augment the ruminant livestock system's sustainability, strategies to reduce emissions while maintaining productivity and ensuring rural livelihoods, need to be implemented. Given the diverse production systems across Latin America, mitigation approaches must be tailored to regional climatic, agroecological, and economic conditions. This abstract provides an overview of mitigation challenges and opportunities in the region, focusing on seven countries - Argentina, Brazil, Chile, Colombia, Paraguay, Peru, and Uruguay - which collectively hold nearly one-quarter of the global cattle population.

Relevance and characterization of livestock production systems

In these seven countries, the livestock sector accounts for 3 to 23% of the agricultural gross domestic product. An average of 17% (4 to 36%) of the population resides in rural areas, where employment is heavily dependent on livestock farming, from small-scale family operations to commercial enterprises. The region is a key global food supplier due to its high production capacity and export-oriented nature. It is home to some of the world's largest cattle herds, with Brazil, Argentina, Paraguay, and Uruguay ranking among the top ten world beef exporters with 3.155kt of deboned beef exported in 2023 (40% of global exports). Pasture-based livestock systems are very relevant and play a crucial role in providing ecosystem services such as soil health preservation, biodiversity conservation, and carbon sequestration. Major unique biomes where cattle farming is practiced serve as important biodiversity reservoirs, such as the Amazon (Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela), Cerrado and Pantanal (Brazil, Bolivia and Paraguay), Atlantic Forest (Argentina, Brazil, and Paraguay), Caatinga (Brazil), Pampa (Argentina, Brazil, and Uruguay) and the Valdivian temperate forest and wetlands (Chile).

Cattle production is the dominant livestock activity across all countries considered, with both beef and dairy sectors playing significant roles. A wide variety of livestock production systems,

ranging from extensive grazing on natural grasslands to more intensive grazing on managed pastures, up to feedlot operations, reflect the region's diverse climates and production approaches. Mixed-integrated production systems also contribute substantially to livestock production. For instance, integrated beef cattle systems combining grazing with feedlot finishing have shown improved productivity while maintaining overall efficiency. Additionally, crop-pasture rotations and silvo-pastoral systems have been implemented across some of these countries as sustainable alternatives for livestock production.

Mitigation strategies for ruminant GHG emissions

The seven countries considered here are signatories to the Paris Agreement, most of them with commitments to net-zero emissions by 2050. National GHG inventories show that the agriculture sector is relevant in the GHG profile of most countries, explaining 8 to 73% of total emissions, and in some cases being the main sector (e.g., Paraguay and Uruguay). The main driver for the sector's emissions is CH₄ particularly from enteric fermentation, followed by N₂O, which are associated with the scale of livestock production.

National Determined Contributions (**NDC**) have been reported, indicating mitigation targets and strategies. While NDCs may not explicitly express targets in terms of emission intensities (i.e., emissions per unit of product), with exception of Uruguay, initiatives in different countries often focus on improving productivity as a pathway to sustainability. Developing silvo-pastoral systems is an alternative approach receiving considerable attention in countries such as Colombia and Peru, with favourable impact on animal production and improved carbon sequestration. Furthermore, crop-livestock-forest integrated systems, which are expanding in Brazil, play an additional role in terms of adaptation to climate change. Reducing deforestation is being actively addressed in the region, while many countries also promote the conservation, restoration and expansion of natural forests.

Research and development on mitigation strategies

National and regional research on mitigation strategies for GHG emissions have been steadily increasing over the past 15 years. Due to the relevance of balancing GHG reduction and livestock productivity, the strategies for CH₄ mitigation in the agenda include those with direct mitigating effects on methanogenesis to decrease absolute emissions, but also production practices and recommendations aimed to lower emission intensity.

Most of the interventions being considered for CH₄ mitigation are those in the global agenda, focusing on nutritional strategies such as improved grazing management, improved pasture quality, strategic supplementation on pasture, as well as the modulation of ruminal fermentation using specific feed ingredients and additives (e.g., tannins, seaweed, oils, nitrates, 3-nitrooxypropanol, etc.) or legume species with adequate tannin content. Additionally, genetic selection is being explored to enhance feed efficiency and lower CH₄ emissions. Integrated systems, such as forestry and livestock production systems (i.e., silvo-pastoral or agroforestry production systems) are being investigated due to their important contribution to reducing emissions by sequestering carbon in forest biomass, avoiding emissions and improving the carbon balance at farm level.

Challenges and opportunities

There are strategies to mitigate GHG emissions available, however, the adoption of these practices faces several restraints. One of the main limitations is that most strategies have been developed for intensive production systems, with higher producing breeds, better quality feeds, and confinement systems. This raises concerns about their direct applicability, effectiveness,

and impact in grazing and more extensive systems, and therefore further research is needed to develop region-specific solutions for GHG reduction.

Regional and international research collaborations are actively engaged in developing strategies to mitigate livestock GHG emissions. These partnerships are essential for leveraging expertise, optimizing research efforts, and avoiding duplication. However, research funding and infrastructure in Latin America remain significantly lower than that in developed countries, limiting progress on feasible and scalable solutions. Addressing this gap is crucial for advancing mitigation efforts. Additionally, the private sector's role in developing and adopting these strategies must be considered, requiring well-structured extension services and clear economic incentives. Overcoming these challenges requires coordinated action among key stakeholders, including farmers, industry leaders, policymakers, and researchers, as effective solutions extend beyond the academic sphere.

Beyond mitigation, providing farmers with tools to adapt to climate change, including capacity building and advanced climate forecasting systems, is essential. Strategies that also enhance the resilience of grass-based production systems offer additional benefits.

Conclusions

Given the economic, social, and environmental importance of livestock in the region, effective mitigation strategies must be tailored to the specific realities of Latin American livestock production systems to ensure their feasibility and impact. Future efforts should focus on scalable, cost-effective solutions that integrate traditional practices with innovative approaches, ensuring both mitigation and the long-term sustainability of the sector. While challenges persist, increased investment in research, policy alignment, and financial support for producers can drive the transition toward a more sustainable livestock sector. Collaboration between governments, research institutions, and the private sector is crucial in achieving long-term emissions reductions while ensuring food security and economic sustainability.

498 Challenges and opportunities for mitigation of greenhouse gas emissions from ruminant livestock in the context of sustainable production in Africa

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Abstract

Africa, a continent rich in livestock resources, holds approximately one-third of the world's livestock population however remains highly vulnerable to the impacts of climate change. Divided into five key regions - North, West, Central, East, and Southern Africa - the continent faces the dual challenge of meeting a rapidly growing demand for livestock products while mitigating greenhouse gas (**GHG**) emissions and enhancing the resilience of its livestock production systems. Livestock play a central role in African societies, contributing between 30% and 80% to the agricultural GDP of various countries, while serving as a source of employment, nutrition, cultural heritage, and food security for millions. With a projected population of 2.2 billion by 2050, the average per capita consumption of meat and milk is expected to rise from 19 kg to 26 kg and from 44 kg to 64 kg, respectively, thereby increasing the environmental footprint of livestock production accordingly. In 2022, the African livestock population included 382 million cattle, 419 million sheep, 50 million goats, and 2.4 billion chickens, with East Africa holding the highest livestock density per hectare. Ethiopia leads the continent with over 68 million cattle, while countries like South Africa, Sudan, Tanzania, and Nigeria are key players in beef production. Livestock systems significantly support food security and rural economies but face increased pressure to perform under changing climatic conditions and resource constraints.

Relevance and characterization of livestock production systems

Livestock production systems in Africa are diverse and operate within different agro-ecological zones that include arid/semi-arid, humid/subhumid, and highland regions. The primary systems include extensive pastoral systems, mixed crop-livestock systems, and increasingly, commercial and intensive production models. Approximately 43% of Africa's landmass supports pastoralism, especially in Sudan, Somalia, and Ethiopia, where pastoral based communities contribute significantly to national GDP. These systems are characterized by low input levels, high dependence on natural pastures, and susceptibility to seasonal variability. Mixed crop-livestock systems dominate in sub-Saharan Africa (**SSA**) and contribute significantly to beef, goat, and dairy production, notably within the Intergovernmental Authority on Development (**IGAD**) region. These systems benefit from synergies such as manure recycling, which contributes up to 23% of nitrogen for crop production. Commercial livestock production, such as feedlots and intensive poultry and pig farming, is more developed in southern Africa, particularly South Africa, where modern feeding and breeding technologies improve production efficiency and off-take rates. However, challenges such as low fertility, high mortality, and poor health management continue to limit productivity, especially within traditional and smallholder livestock systems.

Mitigation or adaptation strategies for ruminant GHG emissions

The increasing demand for livestock-derived products and the need to reduce the sector's environmental footprint present a crucial opportunity for Africa to adopt climate-smart livestock practices. The Agriculture, Forestry and Other Land Use (**AFOLU**) sector in Africa contributed approximately 15% of global GHG emissions from agriculture between 2005 and 2014, with livestock alone in SSA responsible for approximately 0.776 gigatons (**Gt**) of CO₂-equivalent in 2020. Ruminants, particularly cattle, are the primary source, accounting for 96% of emissions largely due to enteric fermentation and manure management. The FAO estimates the region annually emits about 416 million tons of CO₂-equivalent, with beef and dairy production as the largest contributors. A significant portion - 64% - of cattle methane emissions originates from pastoral systems, 34% from mixed systems, and only 1% from feedlots. To mitigate these emissions while enhancing resilience, Africa must prioritize strategies that improve productivity and reduce emissions intensity. These include improving feed digestibility and protein concentration, promoting drought-tolerant crops, enhancing manure management, selecting resilient livestock breeds, and adopting improved grazing practices to boost carbon sequestration. Additionally, rumen manipulation, early warning systems, better breeding objectives to include selection for low GHG emitting animals, and robust animal health management offer promising avenues for sustainable intensification.

Challenges in implementing mitigation or adaptation strategies

Despite these opportunities, several constraints hinder the implementation of effective mitigation and adaptation strategies. A major challenge lies in the lack of accurate baseline data, including detailed livestock activity and feed quality information. Most African countries rely on Tier 1 GHG inventories, which lack the granularity required to evaluate the impacts of intervention strategies adequately. The effectiveness of mitigation efforts varies widely across regions and production systems, requiring tailored, context-specific approaches. Furthermore, limited access to finance, technology, extension services, and technical expertise impedes the scaling-up of successful innovations. The complexity of managing mixed crop-livestock systems, coupled with constrained land access and inadequate infrastructure, adds further layers of difficulty. Additionally, knowledge gaps persist regarding the economic viability and long-term sustainability of proposed interventions. Strengthening livestock systems therefore requires coordinated investment in data generation, capacity building, research, and policy development to ensure that environmental goals align with production and livelihood needs.

Conclusion

In conclusion, a holistic approach combining multiple mitigation and adaptation measures tailored to specific regions and systems holds significant promise for transforming African livestock production. Integrated intervention packages can simultaneously enhance system productivity, reduce GHG emissions intensity, and increase farmer incomes by improving resilience to climatic variability and optimizing feed resource use. Research priorities should focus on filling data gaps, particularly especially for pastoral and agro-pastoral systems prevalent in arid and semi-arid areas, while linking emissions directly with livestock production outputs. Improved methods to measure and report emissions will allow African countries to meet international climate commitments and design more effective policies. The livestock sector remains a cornerstone of African agriculture, offering critical pathways to food security, economic development, and climate resilience. Enhancing herd productivity, improving market access, and implementing sustainable practices will be vital to supporting the livelihoods of millions while achieving environmental sustainability across the continent.

500 Challenges and opportunities for mitigation of greenhouse gas emissions from ruminant livestock in the context of sustainable production in Asia

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Abstract

Asia had the largest cattle, sheep and goat population in the world compared with other continents which included 38.5%, 43.6% and 55.4% of world cattle, sheep and goat population, respectively, in the year 2023. In recent decades, livestock production has dramatically intensified with the global population explosion and dietary changes from plant-based to animal-based food. This is especially the case in Asia, where different structures of livestock production systems in urban (less livestock), peri-urban (intensive-production-dominated) and rural areas (mixed system) can be found. Here, we summarize the current efforts in livestock greenhouse gas (**GHG**) mitigation by Asian countries, with a special focus on East Asia, India, and Central Asia, and discuss the potential challenges and opportunities for methane (**CH₄**) mitigation in the livestock industry.

Characterization of livestock production systems

China and India are the major livestock producers in Asia. China has the largest number of sheep and goats in the world, mainly kept for meat production. China is also the third biggest beef producer in the world, by far the largest in Asia. As the largest country in South Asia, India is the main producer of milk in the world, contributing 23% of global milk production. However, the Indian dairy industry is dominated by smallholder dairy farms that contribute 72% of the country's total milk production. Unlike China and India where limited lands are used for grazing livestock, total pasture areas comprise from 81% (Tajikistan) to 95% (Turkmenistan) of all usable agricultural land in Central Asia. Kazakhstan alone has the fifth largest pastoral area of any country on earth. In Western Asian countries such as Iran, cattle and small ruminant farming is practiced throughout the country. About 90% of small ruminants and 65% of cattle are bred by smallholder farmers in rural areas and in small mixed herds in Iran.

Ruminant GHG emissions

Asia is now the leading regional source of CH₄ from dairy, contributing 27% of the global total, more than the combination of both Europe and North America. In China, the total agricultural GHG emission was estimated to be 1.9 billion tonnes of CO₂ equivalent, accounting for 14% of the country's total emissions. Agricultural methane (**AGM**) emissions in China were 23.4 Tg, with enteric fermentation being the largest source, accounting for 43.9% of the total emissions. Indian agriculture accounts for 658 Tg of CO₂ equivalent and is ranked the second largest emitter of the world's total AGM. Methane emission from Indian livestock is estimated to be 12.7 Tg per annum including 11.6 Tg from enteric fermentation and 1.1 Tg from manure management system. In India, bovines contributed to ~92% emissions while smaller ruminants only contributed to a small proportion of GHG. The total GHG emission from agriculture is estimated to reach 25.5 million metric tons of CO₂ equivalent in Korea in the year 2023. According to the "National Greenhouse Gas Inventory Report", there are four major sectors of

CH₄ emissions in Korea including fossil fuel use (8.7%), livestock farming (21.3%), rice paddy (22.7%), and waste landfill (28.2%). In Japan, the GHG emissions from the agriculture sector was approximately 33.5 million metric tons of CO₂ equivalent. A quarter of Japan's CH₄ emissions are from enteric fermentation and about 18.3% from manure management. The amount of N₂O in Japan was 8.6 million tonnes (CO₂-eq) from the agriculture sector, of which 3.4 million tonnes were derived from manure management. In Central Asia, only a few countries such as Turkey and Kazakhstan have consistently submitted annual national GHG inventory reports to the United Nations Framework Convention on Climate Change, and the agricultural sector shows a continuous rise in emissions from 4.1 to 6.1 Tg of CH₄ per year primarily driven by CH₄ emissions from livestock enteric fermentation in Uzbekistan (29.5%), Turkey (27.5%), and Kazakhstan (18.2%).

Mitigation strategies

In relation to GHG related policy, 11 China departments including the Ministry of Agriculture and Rural Affairs jointly released the Methane Emissions Control Action Plan in 2023, which is the first high level document for CH₄ emissions management and control. The Department of Animal Husbandry and Dairying of India is implementing the National Livestock Mission, which inter alia, includes breed improvement and balanced rationing. Feeding livestock with superior quality balanced ration helps to reduce CH₄ emissions from the livestock. South Korea aims to produce a total CH₄ emission of 19.1 million tons in 2030 compared to 27.4 million tons in 2020 and enhance the deployment of low- CH₄ feed to 30% by 2030. Other policies such as gradually increasing the proportion of livestock manure purification and utilization of ICT equipment for better livestock productivity have also been implemented in South Korea.

Technically, various methods have been applied for CH₄ mitigation from ruminants in Asia, among which the development of feed supplementation strategies such as plant extracts, direct-fed microbials, and seaweed is one of the most effective and direct ways. Plant extract-based feed supplements are being developed in East Asian countries where 6016 extracts were included in the Plant Extract Bank as an easy source to discover beneficial phytochemicals. Almost all seaweeds consumed worldwide are produced in Asia, with 57 percent being produced in China alone, followed by Indonesia, South Korea, and the Philippines. *Asparagopsis taxiformis* harvested from China or Korea was reported to reduce 32.4%-99.3% or 50.1%-51.5% of CH₄ production *in vitro*, however, no study has been conducted to verify its *in vivo* effect in Asia. Computer-aided drug discovery methods or *in silico* docking methods were employed to develop CH₄ inhibitor in China and Korea.

Challenges and opportunities

Global South has overtaken Global North as a livestock CH₄ threat for two reasons. The first is continued population and income growth, which has increased consumer demand for beef and dairy products. The second reason is less obvious but far more important, especially when searching for a solution. Asia lags badly in the modernization of its livestock production systems behind other Global North countries, as there are too many indigenous breeds of cattle being fed on low quality pastures. Collaborations with all stakeholders, including government agencies, the private sector and other non-states entities, are essential to address livestock CH₄ emission in Asia. Identifying opportunities for international cooperation and access to funding through academic and financial institutions are also necessary for developing joint activities towards a sustainable livestock sector.

496 Effect of forage characteristics on enteric methane emission across ruminant classes

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Abstract

Methane is a greenhouse gas (**GHG**) with a global warming potential 27 or 81 times greater than CO₂ over a 100-year or 20-year period, respectively (IPCC, 2021). Livestock contribute 30% of global anthropogenic CH₄ emissions (FAO, 2023), with 90% of global livestock CH₄ emissions from ruminant enteric fermentation (IPCC, 2021). Agricultural CH₄ emissions need to be reduced by 11 to 30% by 2030 and by 24 to 47% by 2050 compared to 2010 levels, to limit global warming to 1.5 °C (IPCC, 2018).

While reducing livestock numbers in high-income countries can contribute to meeting climate targets, ruminants play an essential role in food security and in societal and ecosystem services, particularly in low- and middle-income countries, making this approach less viable (Ianotti et al., 2021; Animal Task Force, 2024). Furthermore, ruminants are particularly adept at converting human-inedible plant material into high quality, human-edible food in the form of milk and meat. Ruminants provide additional benefits, such as grazing on non-arable land, providing traction, and producing manure for fuel and fertilizer (Ripple et al., 2014). A wide variety of strategies to mitigate CH₄ emissions from enteric fermentation by ruminant livestock have been developed, including breeding for low CH₄ emissions (e.g., Van Breukelen et al., 2023); improved husbandry (e.g., increased animal productivity and improved animal health; Hristov et al., 2013a) and nutritional approaches (e.g., dietary supplementation with feed additives or lipids, altering forage types, and increasing forage quality; Hristov et al., 2013b).

There is considerable variation in the nutritional characteristics of forages. A high quality forage is characterized by a high apparent total tract organic matter digestibility (%; **ATTDOM**), a low neutral detergent fibre (**NDF**) content (g/kg DM), and a high crude protein (**CP**) content. Using a meta-analysis approach, Arndt et al. (2022) identified that increased grass quality resulted in a decreased CH₄ intensity (g/kg milk; -13%) while improving animal productivity (daily milk yield; +9%). A key advantage of this strategy is its applicability to all production systems (e.g., feedlot systems, mixed systems, and grassland systems). Additionally, the associated improved fibre digestibility (+15%; Arndt et al., 2022) reduces the amount of manure substrate available for microbial fermentation thereby lowering manure CH₄ emissions (IPCC, 2006). A potential trade-off is that the higher ATTDOM is generally associated with an increased CP content, which leads to increased nitrogen excretion (Montes et al., 2013), and subsequently nitrogenous emissions from urine and faeces (Misselbrook et al., 2005). An additional potential trade-off is that improved forage quality can result in increased intake (+2%, not significant; Arndt et al., 2022) and subsequently, due to the additional substrate available for ruminal fermentation, daily CH₄ production (g/d; +7%, not significant; Arndt et al., 2022).

Although dairy cattle, other cattle (defined as beef cattle and non-lactating dairy cows), and small ruminants might have comparable CH₄-forming pathways in the rumen, they differ considerably in their feed intake levels, rumen morphology, rumen physiology, and rate of digesta breakdown and passage (Van Gastelen et al., 2019a). Consequently, the effectiveness of increased forage quality to mitigate CH₄ emissions might also differ across different ruminant classes. Hence, within the present study, we aimed to quantitatively evaluate the enteric CH₄ mitigation potential of improved forage quality across different ruminant classes.

Meta-analysis

A review of the published literature was performed using Web of Science (Thomas Reuters Science, New York, USA), Scopus (Elsevier, Amsterdam, the Netherlands), and Google Scholar, utilizing all possible combinations of the keywords “forage quality”, “methane” (including all variants, such as “CH₄” and “greenhouse gas”), and “ruminants”. After rejecting publications that reported in vitro experiments only, this literature search resulted in 30 publications related to the effect of forage quality on CH₄ emissions in different ruminant classes. For inclusion in the database, publications needed to fulfil the following criteria: (i) enteric CH₄ emissions were measured directly using respiration chambers, the SF₆-tracer gas technique, or the GreenFeed technique (i.e., not estimated), (ii) the chemical composition and ATTDOM, if not available DM digestibility, of the forages were reported, (iii) data on DMI in combination with CH₄ production (g/d) or on CH₄ yield (g/kg DMI) were available. A total of 27 publications met these selection criteria, of which 6 publications were specifically focused on maize or wheat silage quality. Due to the distinct CH₄-reducing mechanism of maize and wheat silage quality (i.e., higher starch content with maturity) vs. general forage quality (i.e., lower NDF content and higher ATTDOM with maturity), along with limited studies and no new research beyond Van Gastelen et al. (2019b), we excluded these publications.

The descriptive summary of the 21 studies, representing 99 treatment means, describing the effect of forage quality on CH₄ emissions is presented in Table 1 and 2; 12 studies representing 58 treatment means for dairy cattle, 6 studies representing 24 treatment means for other cattle, and 3 studies representing 17 treatment means for small ruminants. Emissions of CH₄ reported in L/d or L/kg DMI were converted to g/d or g/kg DMI assuming a molar weight of 16.04 g/mol and a volume of 22.40 L/mol (Brouwer, 1965). Emissions of CH₄ reported in MJ were converted using 55.65 kJ/g (Brouwer, 1965). If digestible organic matter intake (**DOMI**; kg/d) was not provided, this was calculated from organic matter (**OM**) content of the complete ration, ATTDOM, and DMI. If CH₄ yield (g/kg DMI or g/kg DOMI) was not available, this was calculated from reported or calculated DMI and DOMI, along with daily CH₄ production (g/d).

Table 1. Descriptive summary of the studies used to determine the effect of forage quality on CH₄ emission

Study	Ruminant type ¹	CH ₄ measurement technique ²	F:C ³	Feed allowance	Type of forage
Alvarez et al. (2022)	1	GF	From 50:50 to 80:20	Ad libitum	Silage mixture of timothy, meadow fescue, meadow grass, and white clover
Brask et al. (2013)	1	RC	65:35	Ad libitum	Grass-clover silage
Gordon et al. (1995) ⁴	1	RC	50:50	Ad libitum	Grass silage
Hansen et al. (2021)	1	RC	100:0	Ad libitum	Silage mixture of perennial ryegrass, festulolium, white clover, and red clover
Hansen et al. (2022)	1	RC	95:5	Ad libitum	Herbage mixture of perennial ryegrass and white clover
Lahart et al. (2024)	1	GF	From 80:20 to 90:10	Ad libitum	Perennial ryegrass pasture
Pang et al. (2021)	1	GF	50:50	Ad libitum	Silage mixture of timothy and red clover
Warner et al. (2015)	1	RC	80:20	Restricted	Herbage mixture of perennial ryegrass and timothy
Warner et al. (2016)	1	RC	80:20	Restricted	Silage mixture of perennial ryegrass and timothy
Warner et al. (2017)	1	RC	80:20	Restricted; low and high intake	Silage mixture of perennial ryegrass and timothy
Weiby et al. (2024)	1	GF	67:33	Ad libitum	Timothy silage
Yan et al. (1996)	1	RC	60:40	Ad libitum	Perennial ryegrass silage
Boadi and Wittenberg (2002)	2	SF ₆	100:0	Ad libitum	Mixed hay of legume and grass, or grass hay
Dini et al. (2017)	2	SF ₆	100:0	Ad libitum	Mixed grass and legume pasture
Hart et al. (2009)	2	SF ₆	100:0	Ad libitum	Perennial ryegrass pasture
Jonker et al. (2016)	2	RC	100:0	1.1, 1.5, and 1.8 × maintenance	Grass pasture
Pinares-Patino et al. (2003)	2	SF ₆	100:0	Ad libitum	Timothy herbage
Parnian-Khajejdizaj et al. (2023)	2	RC	100:0	Ad libitum	Perennial ryegrass silage
Armstrong (1964)	3	RC	100:0	1.0 and 2.0 × maintenance	Dried perennial ryegrass, cocksfoot, and timothy
Molano and Clark (2008)	3	SF ₆	100:0	0.75, 1.0, 1.25, 1.50, and 2.0 × maintenance	Perennial ryegrass herbage
Ribeiro et al. (2014)	3	RC	100:0	Ad libitum	Grass silage

¹ According to the IPCC ruminant classes; 1 = dairy cattle, 2 = other cattle (i.e., beef cattle and non-lactating dairy cattle), and 3 = small ruminants.

² GF = GreenFeed, RC = respiration chambers, and SF₆ = SF₆ tracer technique.

³ Forage to concentrate ratio.

⁴ No DOMI reported or possible to calculate DOMI.

Table 2. Descriptive summary of the dairy cattle (n=58 treatment means), other cattle¹ (n=24 treatment means), and small ruminant (n=17 treatment means) data used for the meta-analysis

Item	Mean	Median	Minimum	Maximum
Forage quality characteristics				
CP content (g/kg DM) of forage				
Dairy cattle	164	150	76	286
Other cattle	144	124	44	314
Small ruminants	132	138	54	246
NDF content (g/kg DM) of forage				
Dairy cattle	439	441	288	603
Other cattle	563	561	401	754
Small ruminants	543	515	331	749
OM content (g/kg DM) of forage				
Dairy cattle	914	912	894	935
Other cattle	915	911	876	966
Small ruminants	927	928	885	948
Apparent total tract OM digestibility (%) of complete diet				
Dairy cattle	75.6	77.1	66.0	83.6
Other cattle	63.4	64.9	31.1	83.0
Small ruminants	71.0	76.0	42.3	88.0
Feed intake				
DMI (kg/d)				
Dairy cattle	16.9	16.3	8.0	22.4
Other cattle	7.9	7.3	4.9	12.8
Small ruminants	0.95	0.91	0.73	1.38
DOMI (kg/d)				
Dairy cattle	11.7	11.5	4.9	16.0
Other cattle	4.5	4.3	1.3	8.5
Small ruminants	0.61	0.59	0.48	0.75
CH ₄ emission				
Production (g/d)				
Dairy cattle	368	357	210	511
Other cattle	173	167	106	273
Small ruminants	20.7	21.8	11.7	25.7
Yield (g/kg DMI)				
Dairy cattle	21.8	21.7	15.4	29.6
Other cattle	22.9	22.9	14.3	26.4
Small ruminants	22.4	24.1	10.7	26.5
Yield (g/kg DOMI)				
Dairy cattle	31.8	31.2	21.9	42.4
Other cattle	41.9	38.9	28.9	85.5
Small ruminants	33.9	34.6	21.8	40.8

¹ Beef cattle and non-lactating dairy cattle.

In the meta-analysis, we determined the relation between forage quality characteristics and 2 units of CH₄ yield, namely in g/kg DMI and in g/kg DOMI. When expressing CH₄ emission relative to DMI, multiple factors influence the relationship with forage quality characteristics. These include the amount of OM fermented in the rumen as well as shifts in ruminal fermentation VFA profile associated with increased ATTDOM, as described in more detail

below. These factors modulate the relationship between forage quality characteristics and CH₄ yield (g/kg DMI). By expressing CH₄ emissions relative to DOMI, the amount of OM fermented in the rumen is accounted for and the relationship between forage quality characteristics and CH₄ yield (g/kg DOMI) depends primarily on (1) the shift in ruminal fermentation VFA profile and subsequently H₂ availability for CH₄ production and (2) increased microbial efficiency due to increased fractional passage rate from the rumen, resulting in more of each unit of degraded substrate in the rumen being captured in microbial biomass and less in fermentation end-products (Dijkstra et al. 2007).

To determine the relationship between both units of CH₄ yield and forage quality, a mixed model univariate regression procedure (PROC MIXED of SAS; version 9.4, SAS Institute Inc., Cary, NC) was applied per ruminant class (i.e., dairy cattle, other cattle, and small ruminants), similar to Van Gastelen et al. (2019b). Briefly, this included a random experiment effect, with trials nested within experiment as several experiments had more than one trial, and forage quality variables (i.e., ATTDOM in %, NDF content in g/kg DM, and CP content in g/kg DM) as fixed continuous effects. Adjusted independent variable values were subsequently calculated based on regression parameters of the developed model to determine the R² corrected for experiment effect, as described by St-Pierre et al. (2001). The definition of what is considered to be a weak, moderate, or strong relationship varies considerably in literature. Therefore, to avoid confusion, we considered a relationship to be weak when R² < 0.30, moderate when 0.30 ≤ R² < 0.70, and strong when R² ≥ 0.70.

Results and Discussion

Apparent total tract OM digestibility

The digestibility of OM was weakly negatively related with CH₄ yield (g/kg DMI) in dairy cattle (P = 0.001, R² = 0.22). A strong negative relationship between ATTDOM and CH₄ yield (g/kg DOMI) was observed across all ruminant classes (P < 0.001; R² of 0.83 to 0.94), with slope values indicating a decrease in CH₄ yield (g/kg DOMI) ranging from 0.383 (small ruminants) to 0.959 (other cattle) per 1 percentage point increase in ATTDOM (Table 3, Figure 1).

Table 3. Equations developed to predict CH₄ yield (g/kg DMI and g/kg DOMI) based on apparent total tract OM digestibility (%) of forage

Item	Intercept	SE	P-value	Slope	SE	P-value	R ²
CH ₄ yield (g/kg DMI)							
Dairy cattle	32.8	3.20	< 0.001	-0.146	0.0415	0.001	0.22
Other cattle	24.2	2.37	< 0.001	-0.018	0.0316	0.579	0.01
Small ruminants	17.2	4.49	0.030	0.043	0.0340	0.230	0.31
All ruminants (overall)	23.8	1.67	< 0.001	-0.026	0.0212	0.224	0.03
CH ₄ yield (g/kg DOMI)							
Dairy cattle	84.9	4.62	< 0.001	-0.698	0.0598	< 0.001	0.83
Other cattle	104.3	8.12	< 0.001	-0.959	0.1148	< 0.001	0.92
Small ruminants	57.8	7.19	0.005	-0.383	0.0462	< 0.001	0.94
All ruminants (overall)	86.1	4.19	< 0.001	-0.717	0.0550	< 0.001	0.90

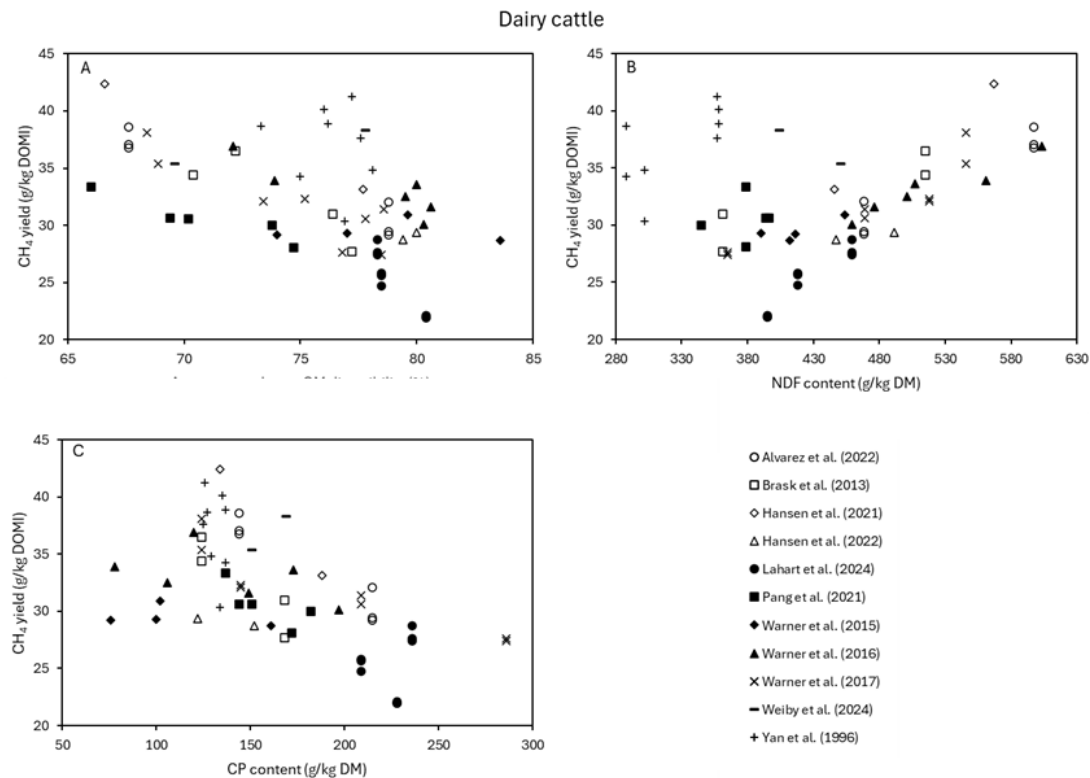


Figure 1. The relationship between methane yield (g/kg DOMI) and forage quality characteristics in dairy cattle; apparent total tract OM digestibility (%) A, NDF content (g/kg DM) B, and CP content (g/kg DM) C. Methane yield (g/kg DOMI) has been corrected.

Increased forage ATTDOM leads to more total tract OM digestion and more OM fermentation in the rumen (e.g., Hansen et al., 2021), which typically increases enteric CH₄ emission per kg feed due to more substrate availability for CH₄ formation. However, increased forage ATTDOM also tends to increase DMI (Van Gastelen et al. 2019a), which generally reduces CH₄ production per unit of feed because of the shorter mean retention time of the feed in the rumen (Moe and Tyrrell, 1979). Moreover, increased ATTDOM tends to accelerate ruminal fermentation and subsequently increases the molar proportion of propionate (Pacheco et al., 2014; Van Lingen et al., 2016), which reduces enteric CH₄ production due to propionate formation being a hydrogen sink.

NDF content

The NDF content (g/kg DM) was moderately positively related with CH₄ yield (g/kg DMI) in dairy cattle ($P < 0.001$, $R^2 = 0.60$). A strong positive relationship was observed between NDF content and CH₄ yield (g/kg DOMI) across all ruminant classes ($P < 0.001$; R^2 of 0.76 to 0.87), with slope values indicating an increase in CH₄ yield (g/kg DOMI) ranging from 0.028 (small ruminants) to 0.088 (other cattle) per 1 g/kg DM increase in NDF content (Table 4, Figure 2).

Table 4. Equations developed to predict CH₄ yield (g/kg DMI and g/kg DOMI) based on NDF content (g/kg DM) of forage

Item	Intercept	SE	P-value	Slope	SE	P-value	R ²
CH ₄ yield (g/kg DMI)							
Dairy cattle	15.6	1.36	< 0.001	0.014	0.0026	< 0.001	0.60
Other cattle	20.9	1.89	< 0.001	0.0037	0.00276	0.201	0.11
Small ruminants	21.5	4.52	0.024	-0.0026	0.00350	0.483	0.14
All ruminants (overall)	19.5	1.15	< 0.001	0.0051	0.00180	0.006	0.29
CH ₄ yield (g/kg DOMI)							
Dairy cattle	10.9	2.65	< 0.001	0.048	0.0051	< 0.001	0.86
Other cattle	-8.3	8.56	0.347	0.088	0.0120	< 0.001	0.87
Small ruminants	16.6	6.79	0.076	0.028	0.006379	< 0.001	0.76
All ruminants (overall)	5.1	3.28	0.125	0.060	0.005393	< 0.001	0.86

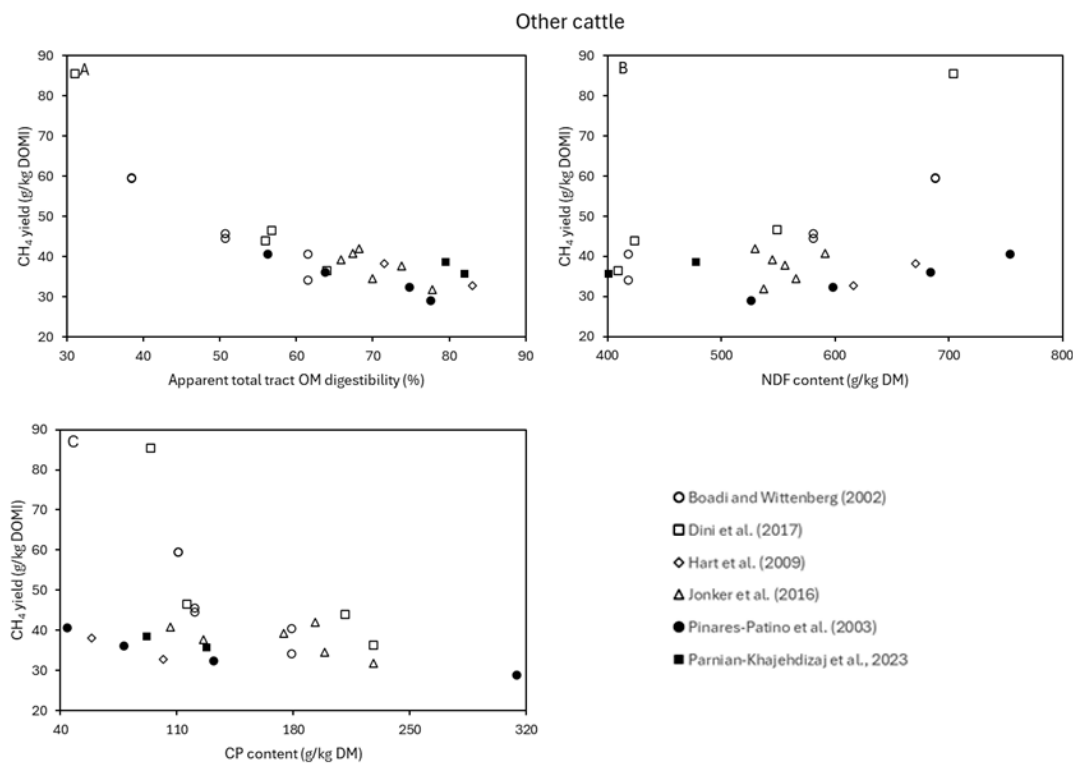


Figure 2. The relationship between methane yield (g/kg DOMI) and forage quality characteristics in other cattle (i.e., beef and non-lactating dairy cattle); apparent total tract OM digestibility (%) A, NDF content (g/kg DM) B, and CP content (g/kg DM) C. Methane yield (g/kg DOMI) has been corrected for study effect. The different symbols identify the 6 individual studies as described in Table 1.

Higher forage ATTDOM is often accompanied by lower NDF concentrations, primarily due to the negative relationship between forage ATTDOM and forage maturity as a consequence of the increased stem-to-leaves ratio of the forage and associated increases in acid detergent fibre (**ADF**) and lignin (Van Gastelen et al., 2019a). Across all data, a decrease of 17 g NDF/kg DM ($P < 0.001$) was accompanied by a 1 percentage-point increase in ATTDOM. Ruminal fermentation of NDF favours the production of acetate and subsequently H₂ availability, activity of rumen methanogens (Van Kessel and Russel, 1996; Hook et al., 2011), ultimately leading to increased CH₄ production.

CP content

The CP content (g/kg DM) was moderately negatively related with CH₄ yield (g/kg DMI) in dairy cattle ($P < 0.001$, $R^2 = 0.57$) and tended to be weakly negatively related with CH₄ yield (g/kg DMI) in other cattle ($P = 0.082$, $R^2 = 0.24$). There was a moderately negative relationship between CP content and CH₄ yield (g/kg DOMI) for all ruminant classes ($P \leq 0.023$; R^2 of 0.34 to 0.72), with slope values indicating a decrease in CH₄ yield (g/kg DOMI) ranging from 0.055 (small ruminants) to 0.085 (other cattle) per 1 g/kg DM increase in CP content (Table 5, Figure 3).

Table 5. Equations developed to predict CH₄ yield (g/kg DMI and g/kg DOMI) based on CP content (g/kg DM) of forage

Item	Intercept	SE	P-value	Slope	SE	P-value	R ²
CH ₄ yield (g/kg DMI)							
Dairy cattle	25.2	0.84	< 0.001	-0.022	0.0038	< 0.001	0.57
Other cattle	24.1	1.20	< 0.001	-0.0079	0.00419	0.082	0.24
Small ruminants	19.9	4.28	0.040	0.0010	0.00559	0.857	0.00
All ruminants (overall)	23.6	0.80	< 0.001	-0.0108	0.002767	< 0.001	0.33
CH ₄ yield (g/kg DOMI)							
Dairy cattle	41.9	1.69	< 0.001	-0.062	0.0084	< 0.001	0.72
Other cattle	53.3	5.98	< 0.001	-0.085	0.03392	0.023	0.34
Small ruminants	39.5	5.53	0.012	-0.055	0.01358	0.001	0.68
All ruminants (overall)	45.6	2.33	< 0.001	-0.072	0.01141	< 0.001	0.52

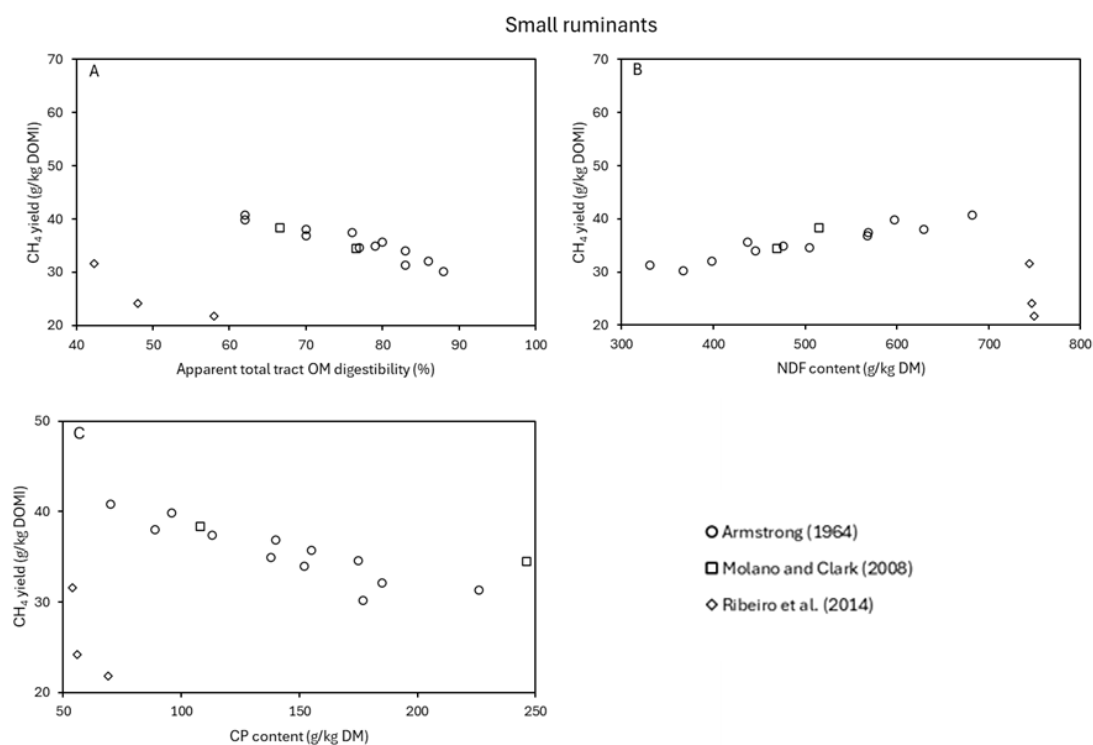


Figure 3. The relationship between methane yield (g/kg DOMI) and forage quality characteristics in small ruminants; apparent total tract OM digestibility (%) A, NDF content (g/kg DM) B, and CP content (g/kg DM) C. Methane yield (g/kg DOMI) has been corrected for study effect. The different symbols identify the 3 individual studies as described in Table 1.

For each unit of rumen-fermented protein, a smaller amount of enteric CH₄ is produced compared with each unit of rumen-fermented sugar and fibre (Bannink et al., 2008). Hence, an increased CP content might be related with a decreased CH₄ yield. Across all data, forage CP content was positively related with forage ATTDOM (i.e., an increase of 12 g CP/kg DM ($P < 0.001$) was accompanied by a 1 percentage-point increase in ATTDOM). The negative relationship between CP content and CH₄ yield (g/kg DMI) is in line with Ellis et al. (2012) and Van Lingen et al. (2018), but contrary to Ellis et al. (2009) who found a positive relationship, as well as to other studies (e.g., Menezes et al., 2016, beef cattle; Niu et al., 2016, dairy cattle) who observed no difference in CH₄ yield with different dietary CP contents. The discrepancies in results between the current and previous studies might be related to the change in dietary CP content. The latter two studies for example achieved differences in dietary CP content by changing the concentrate composition, where only CP was adjusted and all other nutrients remained unchanged. In the present study however, the change in CP is due to differences in forage quality, where a change in forage quality is not only associated with a higher CP content, but also with a higher ATTDOM and lower NDF content.

The CP content is often positively related with forage ATTDOM and negatively related with NDF content (Van Gastelen et al., 2019b). Therefore, it is likely that the relationship between CP content and CH₄ yield in the present analysis may be partially explained by the accompanying change in NDF content and ATTDOM.

Unit of CH₄ emission

The relationship between forage quality characteristics and CH₄ emissions depended on the unit in which CH₄ emission was expressed. In the current study, the relationship between forage quality characteristics (i.e., ATTDOM, NDF content, and CP content) and CH₄ yield (g/kg DMI) was stronger than the relationship between the same forage quality characteristics and CH₄ yield (g/kg DMI). This was rather consistent for all 3 ruminant classes, where the relationship either changed from non-significant or tendency to statistical significance (e.g., other cattle and small ruminants for CP content), or where the significant relationship became stronger (e.g., dairy cattle for ATTDOM).

Ruminant classes: dairy cattle vs. other cattle vs. small ruminants

According to the quantitative meta-analysis of Van Gastelen et al. (2019b), improved grass silage quality consistently decreases CH₄ yield (g/kg DMI) in dairy cattle and was considered to be an effective enteric CH₄ mitigation strategy. The non-quantitative overview by Van Gastelen et al. (2019a) reported that the effect of improved forage quality on enteric CH₄ emission varied among the ruminant classes: dairy cattle (i.e., most effective), beef (not effective), and sheep (not effective or opposite effect). In the present study, we expanded the database by adding more recent publications (from 2018 onwards) and performed a quantitative meta-analysis to determine whether improved forage quality is equally effective in reducing CH₄ emissions from different ruminant classes. For the comparison between ruminant classes, it is important to take note of a number of factors. First, the number of studies and observations differs greatly between the ruminant classes (from 12 studies and 58 treatment means for dairy cattle to 3 studies, and 17 treatment means for small ruminants). Second, the variance in forage quality characteristics varies greatly between ruminant species due to difference in dietary conditions (e.g., minimum ATTDOM for dairy cattle was 66.0% and for other cattle 33.1%; Table 2). Both factors may contribute to the differences observed between ruminant classes. It is furthermore evident that the level of feed intake differs greatly between ruminant classes (Table 2), partially due to experimental design (Table 1), but mostly due to

differences in body size, nutritional requirements, and rumen capacity (Van Gastelen et al., 2019a). The confounding nature of the data prevents a clear determination of how much feed intake contributes to the observed differences across ruminant classes. Warner et al. (2017) observed no interaction between grass quality and level of feed intake in dairy cattle, suggesting that CH₄ emission decreased with improved grass quality irrespective of the level of feed intake. Hence, we expect that the differences between ruminant classes might be caused by a combination of feed intake level and other rumen physiology related factors.

When considering CH₄ yield in g/kg DMI, substantial differences were observed between ruminant classes. For dairy cattle, we found statistically significant relationships between CH₄ yield (g/kg DMI) and the forage quality characteristics ATTDOM (negative; weak), NDF content (positive; moderate), and CP content (negative; moderate). For other cattle, we found only a tendency for a weak positive relationship between CH₄ (g/kg DMI) and CP content. For small ruminants, no statistically significant relationships were detected between CH₄ yield (g/kg DMI) and the forage quality characteristics. Based on these results, one may conclude that improved forage quality effectively reduces CH₄ yield (g/kg DMI) only in dairy cattle. However, when CH₄ emissions were expressed relative to DOMI, the relationships with forage quality characteristics are statistically significant for all ruminant classes, defined as moderate or strong, and always in the expected directions. Based on these results, one may conclude that improved forage quality efficiently reduces CH₄ yield (g/kg DOMI) across ruminant classes.

Combining these results, however, suggests that the shifts in fermentation and H₂ pathways are similar/identical across ruminant classes (evident by overall agreement in effect on CH₄ yield in g/kg DOMI). However, the feed intake level or the extent of fermentation and associated processes such as rate of passage differ considerably between ruminant classes (evident by large differences for CH₄ yield in g/kg DMI). Both cattle classes appear to digest low- to medium-quality forages to a greater extent than small ruminants due to the longer mean retention time in the rumen, whereas all ruminant classes appear to digest high-quality forages equally well (e.g., Soto-Navarro et al., 2014). This suggests that an increase in forage quality enhances ruminal fermentation relatively more in small ruminants than in both cattle classes, resulting in relatively more CH₄ production, unless a shift towards H₂ sinks (i.e., propionate) occurs (Van Gastelen et al., 2019a). As shown in the overview of Van Gastelen et al. (2019a), the increase in DMI relative to BW with improved forage quality was large for dairy cattle, leading to increased ruminal fermentation and subsequently increased H₂ production and concentration that, by thermodynamic control of NADH oxidation, will drive the end products of fermentation toward greater propionate production (Van Lingen et al., 2016). The feed intake of small ruminants was not affected by improved forage quality (Van Gastelen et al., 2019a), resulting in relatively low and unaffected ruminal H₂ production, and consequently no trend toward elevated propionate production. This likely explains the lack of relation between CH₄ yield (g/kg DMI) and forage ATTDOM for small ruminants. For other cattle, an increase in DMI relative to BW with increased forage quality is also observed (Van Gastelen et al., 2019a). It is, however, questionable whether this increase is large to alter ruminal fermentation, H₂ production, and propionate production, considering the overall lack of relation between forage quality and CH₄ yield (g/kg DMI).

As explained above, when expressing CH₄ yield relative to DOMI, the relationship between forage quality characteristics and CH₄ yield (g/kg DOMI) depends primarily on the shift in ruminal fermentation VFA profile and subsequently H₂ availability for CH₄ production, rather than on the amount of OM fermented in the rumen. Increased ATTDOM tends to result in a

faster ruminal fermentation which may be associated with greater molar proportion of propionate in the ruminal VFA produced and more efficient microbial growth (leading to less VFA per unit of OM fermented). Furthermore, the lower NDF content associated with greater forage quality tends to shift the ruminal VFA from acetate to propionate production. The favoured production of propionate reduces H₂ availability to produce CH₄. Ruminal microbial communities can be differentiated by host (i.e., bovines, including dairy and beef cattle, vs. caprids, including sheep), with bacteria, rather than archaea or protozoa, being the main drivers behind these differences (Henderson et al., 2015; Jeyanathan et al., 2011). Methanogens are thus rather similar across ruminant classes, indicating a similar effectiveness if a dietary strategy targets the methanogens directly. Increased forage quality does not directly target the methanogens but affects H₂ availability for methanogens which will likely be similar across ruminant classes, explaining the similar impact of improved forage quality on reducing CH₄ yield (g/kg DOMI). The differences in strength of the relationships between ruminant classes, ranging from moderate to strong, might be a consequence of different fermentation conditions, which depend, among others, on feeding level, rumen size, turnover of rumen content, microbial growth rate, and microbial community.

Conclusion

Improved forage quality is characterised by increased ATTDOM and CP content, and a decreased NDF content. Based on the meta-analysis conducted, we conclude that the effectiveness of reducing enteric CH₄ emissions by improved forage quality differs across ruminant classes when CH₄ is expressed relative to DMI, being effective in dairy cattle, but not in other cattle or small ruminants. When CH₄ emission is expressed relative to DOMI, improved forage quality effectively reduces enteric CH₄ emission across all ruminant classes, with ATTDOM and CP content negatively related and NDF content positively with CH₄ yield (g/kg DOMI).

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480 Assessing the climate footprint of agricultural systems in the tropics: carbon sequestration and GHG emissions

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Abstract

Agricultural systems in tropical regions, including those in Sub-Saharan Africa, Latin America, Southeast Asia and the Pacific, play a dual role in contributing to and being affected by climate change. These systems are significant sources of greenhouse gas (GHG) emissions while also substantially storing carbon. Accurate assessment of their climate footprint is essential for enhancing global Earth system models and informing regionally adapted mitigation and adaptation strategies. This review provides a comprehensive overview of methods used to quantify both GHG emissions and carbon storage across diverse tropical agricultural landscapes. Conventional approaches, such as life cycle assessment (LCA), ecosystem-based analysis, and simulation modelling, remain foundational, yet they are increasingly supported by advanced monitoring technologies, including remote sensing and geospatial tools. Direct measurement techniques, such as eddy covariance flux towers, static and dynamic chamber methods, respiration chambers, GreenFeed systems, SF₆ tracer techniques, and spectroscopic methods, offer high-resolution data on emissions from soil, surface water, crop, livestock, forestry, and agroforestry systems. Integration of these technologies enables improved precision, spatial scalability, and temporal resolution in climate footprint assessments. However, implementation is often constrained by infrastructural limitations, data scarcity, financial barriers, and weak institutional frameworks, particularly in low- and middle-income tropical countries. Addressing these constraints through targeted policy support, investment in scientific infrastructure, and regional capacity building is essential for unlocking the mitigation and adaptation potential of tropical agriculture. This review identifies critical gaps and highlights opportunities to strengthen climate footprint assessments in tropical agricultural systems worldwide.

Introduction

Agricultural systems in tropical regions including Sub-Saharan Africa, Latin America, Southeast Asia, and the Pacific are essential to global food security, rural livelihoods, and socio-economic development. These systems, largely driven by smallholder farming, provide employment for hundreds of millions and are central to national economies. However, they are also under increasing pressure to meet the demands of a growing population while addressing environmental degradation and climate change. Agriculture in the tropics is a significant anthropogenic source of GHG emissions, primarily through deforestation, land-use change, livestock production, and soil degradation.

Emissions originate mainly from three processes: methane (CH₄) released through enteric fermentation in ruminants, nitrous oxide (N₂O) from synthetic and organic fertilizer use on croplands, and a combination of CH₄ and N₂O from irrigated systems. In addition, carbon

dioxide (**CO₂**) emissions result from forest conversion, peatland drainage, and soil degradation (Tubiello et al., 2021). At the same time, tropical agriculture possesses significant potential to contribute to climate change mitigation through soil carbon sequestration, reforestation, agroforestry, and improved pasture management. Yet, the sector remains highly vulnerable to changing climatic conditions, with cascading effects on food production, rural economies, and ecological stability.

In this context, accurately assessing the climate footprint of agricultural systems encompassing both GHG emissions and carbon storing is essential for identifying effective mitigation strategies, supporting adaptation planning, and informing national climate policies. Climate footprint assessments are also crucial for countries seeking to access climate finance, meet Nationally Determined Contributions (**NDCs**), and participate in carbon markets. Despite growing political and scientific interest, many tropical countries face persistent challenges: limited access to high-resolution data, inadequate measurement infrastructure, weak modelling capacity, and underrepresentation in global GHG inventories.

Moreover, the diversity of agricultural systems across tropical regions ranging from traditional subsistence farming to commercial monocultures adds complexity to GHG assessment and carbon accounting. In many cases, methodological limitations, lack of standardized protocols, and insufficient investment in monitoring and reporting systems hinder robust evaluation of agriculture's climate impact.

This review aims to provide a comprehensive overview of current methods used to assess the climate footprint of tropical agricultural systems, with a dual focus on GHG emissions and carbon storing. We explore conventional approaches, evaluate their strengths and limitations, and discuss the ecological processes, land-use dynamics, and GHG fluxes associated with tropical agriculture.

Tools for GHG inventory and carbon storage evaluation of agricultural systems

IPCC software for national GHG inventories

The intergovernmental panel on climate change (**IPCC**) provides standardized methodologies and software tools to support countries in reporting their ghg emissions under the united nation framework convention on climate change (**UNFCCC**). The IPCC inventory software integrates tier 1, tiers 2 and tier 3 methods based on default (tier 1) or country-specific emission factors (tier 2, tier 3) combined with activity data (IPCC, 2019). For agriculture, this includes modules for enteric fermentation, manure management, agricultural soils, rice cultivation and field burning of crop residues.

GLEAM: global livestock environmental assessment model

GLEAM (developed by FAO) is a spatially explicit model that estimates GHG emissions along livestock supply chains, covering production to processing. It integrates data on herd structures, feed intake, manure management, and production systems at global and regional levels (Gerber et al., 2013). GLEAM uses a Tier 2/IPCC approach.

EX-ACT

The Ex-ante carbon-balance tool (**EX-ACT**), developed by FAO, is widely recognized as an effective methodology for assessing the climate impact of agricultural interventions. Designed to estimate changes in ghg emissions and carbon storage, it is particularly suitable for

application in sub-Saharan Africa (**SSA**), where data limitations and diverse agro-ecological systems require adaptable and accessible tools.

Process-Based Biogeochemical Models, STEP-GENDEC-N₂O

Process-based biogeochemical models simulate soil-plant-atmosphere processes and are widely used to estimate GHG emissions from terrestrial ecosystems. These models link ecological drivers to GHG emissions and serve as convenient tools for studying, quantifying, and predicting how changes in these drivers may influence emissions. They are often used to complement field experiments and to extrapolate findings to broader spatial and temporal scales. In SSA, recent studies have applied process-based models to estimate GHG (CO₂ and N₂O) emissions at both local and regional scales. For example, the STEP-GENDEC-N₂O model (Agbohessou et al. 2023; Bigaignon et al., 2020; Delon et al., 2019; Moorhead and Reynolds, 1991; Mougin et al., 1995), a coupled, process-based model specifically developed for Sahelian herbaceous savannas, simulates the cycling of water, C, and N between the atmosphere, vegetation and soil. This model has recently been used to estimate CO₂ and N₂O emissions from silvopastoral systems at local scales in west Africa (Agbohessou et al. 2023; Bigaignon et al. 2020; Delon et al. 2017), as well as at the regional scale across the western Sahel (Agbohessou et al., 2024).

Other process-based models, such as DNDC (Giltrap et al. 2010) and STICS (Brisson et al., 2002, Plaza-Bonilla et al. 2017), have been used to estimate GHG emissions in cropping systems outside SSA.

Regional case studies from Africa and Latin America

This section presents selected case studies from tropical regions - West Africa and South America- highlighting the biophysical complexity and climate impact of agricultural systems. These examples integrate in-situ measurements, ecosystem modelling, and long-term monitoring to derive insights on both GHG sources and carbon sequestration opportunities.

West Africa

a) Widou: Assouma et al. (2018, 2019) conducted a comprehensive study in the Ferlo region of northern Senegal, an open rangeland ecosystem representative of Sahelian pastoralism. The study employed an ecosystem-based approach to quantify the carbon balance of this pastoral system. All major sources of GHG emissions were accounted for, including enteric CH₄ from ruminants, emissions from dung, soil, surface water bodies, termite activity, bushfires, and motorized water pumps. Simultaneously, all relevant carbon sequestration pathways were included, such as carbon storage in soil and woody vegetation biomass. To capture the effects of seasonal variability inherent to the monsoon-driven climate and herd mobility, carbon fluxes were monitored on a monthly basis. The study concluded that the annual carbon balance of the pastoral ecosystem was -0.01 tCO₂eq/ha/yr. This shows that total GHG emissions were mitigated by carbon accumulation in trees, soil and livestock. The carbon balance varied considerably with the seasons. This high seasonality is explained by both pastoral practices and environmental factors. The negative carbon balance found in this study contrasts with the traditional reputation of African livestock systems having a negative impact on climate change, because of their low productivity per animal.

b) Dahra: In a semi-arid silvopastoral system (**SPS**) located in northwestern Senegal, Agbohessou et al. (2023) employed a modelling approach to assess the potential of trees to mitigate GHG emissions in a representative Sahelian SPS. They applied the process-based

STEP-GENDEC-N₂O model (Agbohessou et al. 2023, Bigaignon et al., 2020; Delon et al., 2019; Mougin et al., 1995), designed for Sahelian herbaceous savannas, to simulate soil CO₂ and N₂O emissions and assess the carbon balance of the herbaceous layer. In parallel, they used the agroforestry tree growth model DynACof (Vezy et al. 2020) to quantify the carbon dynamics associated with the tree layer. They calibrated both models using in situ flux measurements of CO₂ and N₂O. They estimated CH₄ emissions from enteric fermentation using the IPCC tier 1 FAO dataset. Their simulations were conducted under two scenarios: (1) the current site conditions, reflecting actual tree density and animal load, and (2) a hypothetical scenario excluding livestock. This dual-scenario framework allowed for the isolation and quantification of the role trees play in mitigating ghg emissions associated with livestock production. Their results showed that, under current conditions, the tree component of the SPS partially offsets livestock-related ghg emissions and may have the potential to fully offset them under optimized conditions. These findings highlight the significant mitigation potential of integrated tree-livestock systems and underscore the importance of managing tree cover and animal load to improve the carbon balance and long-term sustainability of silvopastoral systems.

c) Groundnut basin in Senegal: In the emblematic Sahelian *Faidherbia albida* parklands (Groundnut basin, Sob, 14°29'44.916"N; 16°27'12.851"W; FLUXNET ID: SN-Nkr), net CO₂ exchanges between the soil and the atmosphere were monitored over a full year, encompassing both dry and rainy seasons. The study used two complementary methods: (i) high-frequency automated static chambers, and (ii) the Eddy Covariance (**EC**) technique installed on a flux tower (20m of height), approximately 12.5m above tree canopies. The site has a sandy soil texture and represents a typical 'bush field', characterized by low soil fertility, no mineral fertilization supply, and off-site export of crop residues and manure to home-fields (Malou et al., 2021).

CO₂ fluxes from the soil to atmosphere were continuously monitored using an automated static chamber (n=8) coupled to a Picarro G2508 multigas analyser. Concurrently, the EC technique was used to quantify the net carbon exchange at the ecosystem scale. Measurements were complemented by modelling approaches for soil respiration and photosynthesis fluxes, according to Lasslop et al. (2010) and Lloyd and Taylor (1994).

The results revealed a pronounced "fertile island effect" (Eldridge et al., 2024), characterised by significantly higher fluxes for both soil respiration (CO₂ emission) and photosynthetic activity under the trees (CO₂ uptake by crops). Tree compartments accounted for approximately 50% of the annual gross primary productivity (GPP) at the parkland scale. The system was identified as an effective carbon sink, with net carbon balances of $-1.4 \pm 0.02 \text{ Mg C-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ (upscaled chamber method) and $-1.8 \pm 0.01 \text{ Mg C-CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ (EC method).

This study provides robust dataset for carbon fluxes monitoring in Sahelian regions, highlighting the potential role of *F. albida*-based agroforestry systems for carbon sequestration.

d) Dantchandou: Positioned between conventional approaches, namely, carbon storage ecosystem balance and life cycle assessment, the ongoing thesis of Yanoussa Yakoubou Alzouma investigates the annual contribution of livestock breeding to the net climate balance within an agro-pastoral territory. Specifically, the study focuses on the commune of Dantchandou in Niger, spanning 919 km², to assess how livestock systems influence greenhouse gas emissions and carbon sequestration over a year cycle. The soil CO₂, CH₄,

and N₂O emissions have been measured using portable analyser (CO₂ only) and static chamber in 32 sites representative of the main land uses including manured and unmanured crop fields, fallows and rangelands and livestock concentration spots such as corrals, vicinity of pastoral well and ponds. The monthly GHG emissions were aggregated on an annual basis and weighted by the surface area of each land use type and livestock concentration zone, resulting in territorial-scale estimates of 10.8 Mg CO₂ ha⁻¹ yr⁻¹. The contrast in soil GHG emission between soils receiving or not livestock excretions was used to attribute 21.3% of the CO₂ and negligible amount of CH₄ and N₂O emissions to livestock. A detailed monthly inventory of cattle, sheep, and goats, categorized by sex and age class and managed by agro-pastoralists and farming families within the territory (Alzouma et al., 2024), enabled the estimation of enteric CH₄ emissions using an equation based on dry matter forage intake (Gbenou et al., 2024), which varies according to the animals' monthly metabolic weight. Both feed intake and metabolic weight were extrapolated from a sub-sample of animals and the NIRs analysis of systematically collected animal feces. Aggregated for all the animals present in Dantchandou over the year the CH₄ enteric emission reached 5 MgC ha⁻¹. The annual C balance will be established between the livestock contribution to GHG emissions (soil + enteric) and its contribution to the storage of carbon by the vegetation growing on the lands receiving fecal excretions.

South America (Amazonian pastoral systems)

In the Amazon region, livestock expansion has historically contributed to extensive deforestation, with approximately 80% of cleared forest areas converted to pasture, resulting in rapid carbon emissions (Vigne et al., 2016). However, recent studies in French Guiana and the Brazilian Amazon reveal significant mitigation potential through improved pasture management and spatially informed intensification strategies.

In French Guiana, long-term research combining chronosequence sampling down to 1 m depth with eddy covariance flux tower measurements demonstrated that well-managed tropical pastures (≥ 24 years old) could sequester an average of 1.27 ± 0.37 tC ha⁻¹ yr⁻¹, compared to 3.23 ± 0.65 tC ha⁻¹ yr⁻¹ in native forest soils (Stahl et al., 2017). Practices such as rotational grazing, legume-grass mixtures, and the avoidance of fire and overgrazing were associated with enhanced carbon recovery and sustained soil fertility. Incorporation of these findings into the AgriClimateChange Tool (ACCT) showed that under stable land-use conditions (i.e., no further deforestation), the annual soil carbon sequestration could offset up to 80% of farm-scale GHG emissions (Dallaporta et al., 2016).

In the Brazilian Eastern Amazon, particularly in the municipality of Paragominas, studies based on spatial modelling, remote sensing, and socio-technical surveys revealed that moderate intensification of pastureland use can contribute not only to increased productivity but also to forest regeneration on marginal lands (Osis et al., 2019). These results highlight the importance of considering land suitability for defining intensification zones, while allowing low-potential areas to revert to forest cover. Furthermore, livestock carbon balances must incorporate both pasture dynamics and forest recovery trajectories to accurately assess net GHG outcomes. Moderate intensification, as opposed to high-tech solutions, proved more inclusive, enabling wider adoption even under suboptimal innovation and resource conditions.

Together, these studies confirm that tropical pasture systems in the Amazon, when sustainably managed, can function as partial carbon sinks, contributing significantly to climate mitigation targets under NDCs (de Oliveira Silva et al., 2018).

Conclusion and perspectives

Tropical agricultural systems are central to global climate mitigation and food security goals. This review has emphasized that beyond technological sophistication, climate footprint assessments must embrace the complexity of tropical ecosystems accounting for variability in soils, rainfall, vegetation, and farming practices. A shift toward ecosystem-based approaches that integrate measurements, modelling, and socio-economic realities is urgently needed.

In conclusion, carbon sequestration and GHG mitigation are attainable in tropical agriculture through context-aware practices, ecosystem stewardship, and evidence-based policy integration. Accurately assessing the climate footprint of tropical agricultural systems is a critical step toward developing effective mitigation and adaptation strategies.

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494 Exhaled breath to assess rumen physiology in dairy cows

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Abstract

Ruminal fermentation is the cornerstone of nutrient metabolism in dairy cattle, converting dietary substrates into volatile fatty acids (VFA) – the primary energy sources for the host animal – while concurrently producing greenhouse gases, predominantly methane (CH₄). Enhanced understanding of rumen metabolic processes is critical, given their central role in optimizing dairy productivity and addressing environmental sustainability concerns. Conventional approaches for assessing rumen fermentation, such as rumen cannulation, rumenocentesis, oro-ruminal tubing, and the deployment of rumination boluses, have significantly contributed to advancing knowledge of rumen function, microbial interactions, and nutrient utilization (de Assis Lage et al., 2020; Larsen et al., 2020; Indugu et al., 2021). However, these traditional methods are inherently invasive, labor-intensive, and generally unsuitable for continuous, real-time monitoring, particularly when scaled across large herds or production systems (Duffield et al., 2004; Muizelaar et al., 2020). Recent advances in exhalomics – the study of volatile organic compounds (VOCs) present in exhaled breath – have opened promising avenues as non-invasive alternatives for real-time monitoring of both ruminal fermentation dynamics and overall metabolic health in dairy cows (Islam et al., 2023; Islam et al., 2024a; Haddadi et al., 2022). This emergent field leverages sophisticated analytical methodologies, enabling researchers to detect nuanced metabolic signatures through breath analysis (Lan et al., 2020; Haddadi et al., 2022). The current review explores historical progress, technological developments, key biomarkers, practical applications, and existing challenges associated with the use of exhaled breath analysis to assess rumen fermentation and metabolism in dairy cows.

Evolution of exhaled breath analysis to assess rumen fermentation

The study of exhaled breath as a tool for assessing rumen fermentation began with an interest in non-invasive techniques to monitor metabolic processes in ruminants. Early studies focused on measuring headspace gases, such as CH₄ and carbon dioxide (CO₂), in fistulated animals, where these gases served as proxies for ruminal fermentation efficiency and microbial activity (Aii et al., 1980). Throughout the 1980s and 1990s, scientific investigations primarily centered on the relationship between specific VOCs in ruminal headspace gases and the overall fermentation process in the rumen, although technological limitations restricted detailed analyses (Dobbelaar et al., 1996; Elliott-Martin et al., 1997).

Advances in gas chromatography and mass spectrometry in the early 2000s improved the precise quantification of compounds such as VFA, CH₄, hydrogen (H₂), and other fermentation by-products. For example, Dewhurst et al. (2001) employed selected-ion-flow-tube mass spectrometry (SIFT-MS) to measure gases such as hydrogen sulfide, methyl sulfide, and ammonia (NH₃) in the rumen headspace of fistulated Holstein cows. They observed that sulfur compounds increased and decreased after feeding, a reflection of sulfur amino acid fermentation, while NH₃ concentrations were pH-dependent and could help diagnose subacute ruminal acidosis. On the contrary, the profile of VFA in the headspace of rumen gas showed slight differences from rumen liquor. Following this, researchers began to explore the potential of using ruminal headspace gases to assess dietary and microbial shifts during the rumen

fermentation (Cai et al., 2006; Dewhurst et al., 2007). Specifically, Cai et al. (2006) investigated rumen gas composition in three rumen-cannulated Angus steers using solid-phase microextraction (**SPME**) and gas chromatography-mass spectrometry-olfactometry. The authors identified 50 VOCs, including sulfides, VFA, ketones, and phenolic compounds in rumen gas samples. The abundance of these VOCs and odors, particularly sulfides and VFA, was higher after feeding, indicating that rumen gases have the potential to be used to monitor digestion and fermentation patterns in cows. Dewhurst et al. (2007) also identified interactions between rumen microbial processes (sulfur amino acid degradation) and plant defence mechanisms (cyanogenesis from white clover) in affecting rumen headspace gases. Fonseca et al. (2013) proposed the use of rumen headspace gas concentrations of hydrogen sulfide as a better alternative to assess the key attribute of rumen protein degradation in feeds where high solubility negates the use of the traditional nylon bag in sacco technique.

Until 2018, most studies focused on cannulated animals, by investigating headspace gases and compounds. A major innovation occurred in 2018 when Oertel et al. (2018) introduced a face mask coupled with proton transfer reaction-time of flight-mass spectrometry (**PTR-TOF-MS**) to capture real-time eructation gases. In a pilot study involving cattle, goats, and sheep, the researchers detected up to 36-fold increase in certain VOCs during eructation events in cattle compared to other ruminant species. They also developed a “*Burp Tracker*” algorithm to effectively separate eructation events from normal breathing cycles, to enable more accurate VOC analysis. More recently, Islam et al. (2023) used GreenFeed, a head-chamber system, to monitor CH₄ during eructation events of dairy cows in real-time (Figure 1). This method leveraged CH₄ as a marker to precisely identify burping of cows for the collection of exhalome (total volatile metabolites contained in exhaled breath) samples. The exhalome samples were analyzed off-line via secondary electrospray ionization high-resolution mass spectrometry (**SESI-HRMS**) primarily to quantify ruminal VFA profile. They were able to characterize the diurnal patterns of exhaled VFA in relation to ruminal CH₄ emission pattern from 4 Brown Swiss and 3 Holstein cows (Islam et al., 2023). While a total of 1,298 features were recorded (649 features in positive ion mode and 649 features in negative ion mode), the authors observed that concentrations of exhaled acetate, propionate, and butyrate peaked after feeding and followed a pattern similar to CH₄ production. Moreover, the acetate was the most abundant VFA, followed by propionate and butyrate, reflecting the known ruminal VFA composition.

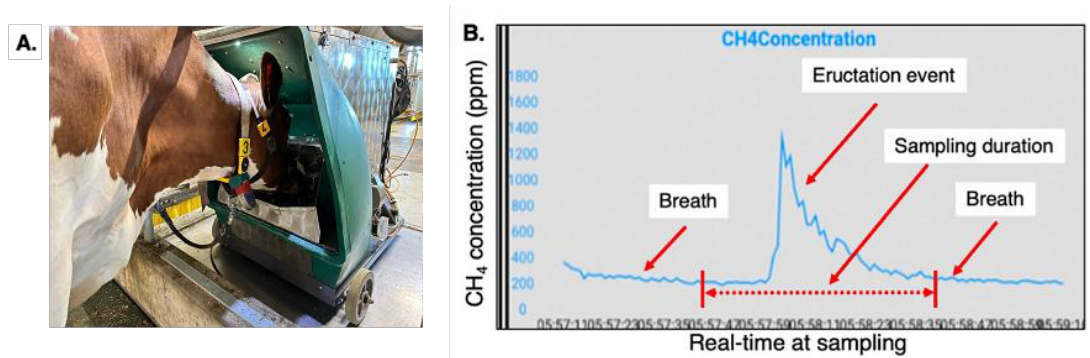


Figure 1. (A) Exhalome sampling of dairy cows using gas sampling unit of the GreenFeed system (C-Lock Technology Inc.). (B) Real-time ruminal CH₄ emission measurements (x-axis: real time of measurement; y-axis: CH₄ concentration, ppm) on the “Control Feed” mobile application (version 1.51, C-Lock Technology Inc.), showing the eructation event with an exhalome sampling duration of approximately 1 min. (Revised from Islam et al., 2023)

Subsequent work by Islam et al. (2024a) validated potency of exhalomics as a non-invasive modality for assessing ruminal fermentation dynamics in dairy cows. Using a switch-back design with 4 cannulated Original Brown Swiss cows subjected to two distinct diets (high-starch vs. low-starch), the authors compared the molar proportions of VFA in exhalome samples with direct rumen samples obtained through the cannula, as well as to the Henry's Law constant predicted VFA profile in the gas phase of the rumen. Exhalome samples were collected over 8 sampling events spanning 24 hours to represent every 3-h of a day, alongside simultaneous rumen fluid sampling from the cannulated cows. The study found no interactions between diet and VFA measurement methods and strong correlations between exhaled and ruminal VFA, with diurnal patterns closely mirroring each other, both peaking post-feeding (Figure 2). These investigations revealed no significant interactions, implying consistent daily VFA molar proportion trajectories, regardless of dietary input. In a complementary approach, Jorge-Smeding et al. (2023) applied SIFT-MS to distinguish cows on high-fiber and high-starch diets. Their work identified exhalome biomarkers linked to sub-acute ruminal acidosis and other diet-induced dysfunctions, further demonstrating that exhalome biomarkers can reflect dietary-induced changes in rumen metabolism.

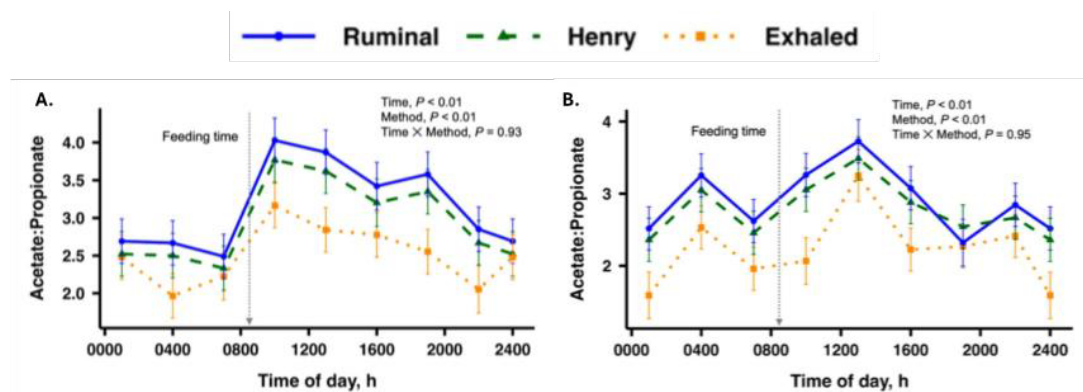


Figure 2. Molar proportions of acetate: propionate ratio measured in dairy cows fed a relatively (A) high-starch diet (16.2% of DM) and (B) low-starch diet (6.31% of DM). (Revised from Islam et al., 2024a)

Beyond methane and VFAs, rumen-derived VOC emissions encompass a broad range of compounds including alcohols, esters, aldehydes, terpenes, phenolics, and sulfur compounds, reflecting diverse microbial and fermentation pathways. Ethanol and short-chain alcohols, for instance, typically indicate anaerobic fermentation, while sulfur-based VOCs such as hydrogen sulfide and dimethyl sulfide potentially signal dysbiosis or dietary imbalances (Akin et al., 1990; Gruninger et al., 2019; Eichinger et al., 2024) in addition to excess rumen degradable amino acids (Dewhurst et al., 2007). Additionally, ammonia levels directly reflect microbial nitrogen metabolism linked to dietary protein degradation (Pellikaan et al., 2011). Recent work integrating the exhalome with rumen fluid metabolomics has illustrated diet-induced metabolic shifts clearly traceable via breath signatures (Islam et al., 2024b).

Challenges, future directions, and concluding remarks

Despite substantial advances, several critical challenges remain before exhalomics can achieve widespread adoption. One primary concern is standardization of breath sampling methodologies; variations across head-chamber systems, face masks, and electronic noses contribute significantly to data variability. Furthermore, accurate discrimination between exhaled gases, from the rumen or host metabolism, and environmental contaminants remains

a technical hurdle. Breath at the nostrils is a complex and rapidly changing mixture of ambient air, eructed rumen gas, and gases from the bloodstream (via exchanges in the lungs). Some breath components are produced by both rumen microbial metabolism and animal metabolism (e.g. dimethyl sulfide; Dewhurst et al., 2001; Elliot-Martin et al., 1997). Breath of grazing ruminants also contains products of plant metabolism, notably the plant defence mechanisms resulting in 'green odor' compounds such as methanol, hexenal and hexenyl acetate which are produced rapidly in response to tissue damage (Kirstine et al., 1998; RJ Dewhurst unpublished observations). Variability in analytical platforms, ranging from online PTR-TOF-MS methods to offline techniques (GC-MS, SIFT-MS, SESI-MS), coupled with limited comprehensive spectral libraries and annotation standards, significantly complicates metabolite identification and interpretation. The relationship between rates of production and concentrations of components of rumen fluid and concentrations in rumen gas is not straightforward, for example being affected by pH in the case of ionic compounds. Changes in pH within the normal rumen range have a major effect on the relative proportions of NH_3 and NH_4^+ . Having said that, the situation for breath analysis is no different to conventional analysis of rumen fluid (e.g. for VFA), which mostly considers concentrations and may not align fully with rates of production (Sutton et al., 2003).

Advances in hybrid approaches combining condensed breath collection with high-resolution metabolomics offer potential solutions; however, numerous metabolites remain unidentified due to incomplete database annotations (e.g., HMDB, KEGG). Network-based visualization and artificial intelligence (AI)-driven annotation methods present promising avenues to navigate complex data interpretation challenges.

Future research priorities should focus on integrating exhalomics with rumen microbiome profiling (metagenomics, metatranscriptomics) to unravel links between specific VOC biomarkers and microbial functional pathways. The development of affordable, portable analytical platforms suitable for on-farm use will significantly enhance practical implementation, facilitating regular metabolic monitoring and herd management decisions. Longitudinal studies establishing baseline VOC biomarker profiles throughout lactation cycles will be critical for predictive diagnostics and early detection of metabolic dysfunctions. Moreover, advancements in AI and machine learning algorithms will be essential for further progress in deciphering complex breathomic datasets, discovering novel biomarkers, and refining metabolic diagnostics.

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495 Animal health as a climate solution

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Abstract

The livestock sector contributes approximately 12% of global anthropogenic greenhouse gas (GHG) emissions (FAO, 2023) with a significant proportion from methane (CH₄) emissions from enteric fermentation and CH₄ and nitrous oxide (N₂O) emissions from manure management. While established mitigation strategies such as feed additives (Hristov, 2024), increased reproductive efficiency (Legesse et al., 2018), selective breeding for low-emission animals (Manzanilla-Pech et al., 2021), and vaccines targeting methanogenic microbes (Wedlock et al., 2013) have received substantial attention, the critical role of animal health in mitigating GHG emissions has been, until recently, largely underexplored. Diseases impair productivity, increase resource use, and prolong production cycles, thereby increasing GHG emissions per kilogram of livestock products (Emissions intensity; EI) such as milk, meat and animal fibres (Capper and Williams, 2023). Improving livestock health offers a strategic and integrated approach to reducing GHG emissions, while simultaneously enhancing animal welfare, farm productivity, food security and enhanced community resilience, and lowering the risk of zoonotic disease transmission (Drieux et al., 2021; Özkan et al., 2022; Kyriazakis et al., 2024).

This paper explores the complex interplay between animal health and GHG emissions, drawing on case studies, modelling insights, and policy developments to highlight the co-benefits of addressing health challenges in livestock systems in the context of climate change. Focusing on key diseases such as gastrointestinal parasitism, mastitis, and infertility and reproductive losses, we examine the significant impact of animal health interventions on reducing GHG EI and potentially in some cases on absolute emissions. Additionally, we address the challenges of modelling disease impacts on GHG EI, particularly in low- and middle-income countries (LMICs), where there is often a lack of quality data on disease prevalence and impacts, veterinary infrastructure is often limited, and farm management practices vary widely. Finally, we discuss the policy implications of integrating animal health into national climate strategies and the critical role of climate finance in advancing health-based interventions.

Livestock health conditions and greenhouse gas emissions

Diseases in livestock systems directly and indirectly increase EI by impairing reproductive performance, productivity and longevity. This relationship is evident across diverse systems, where health issues contribute to slower growth rates, reduced fertility, and lower milk yields, wool and fibre production while increasing mortality and culling rates, and prolonging calving interval (MacLeod et al., 2018; Özkan Gülzari et al., 2018; Mostert et al., 2019). The inefficiencies caused by diseases extend to resource use, with affected animals requiring more feed, water, and energy over prolonged production cycles and the maintenance of increased numbers of replacement animals (Figure 1). These impacts are particularly relevant in ruminants, where CH₄ EI rises sharply with reduced feed efficiency (Johnson et al., 2022).

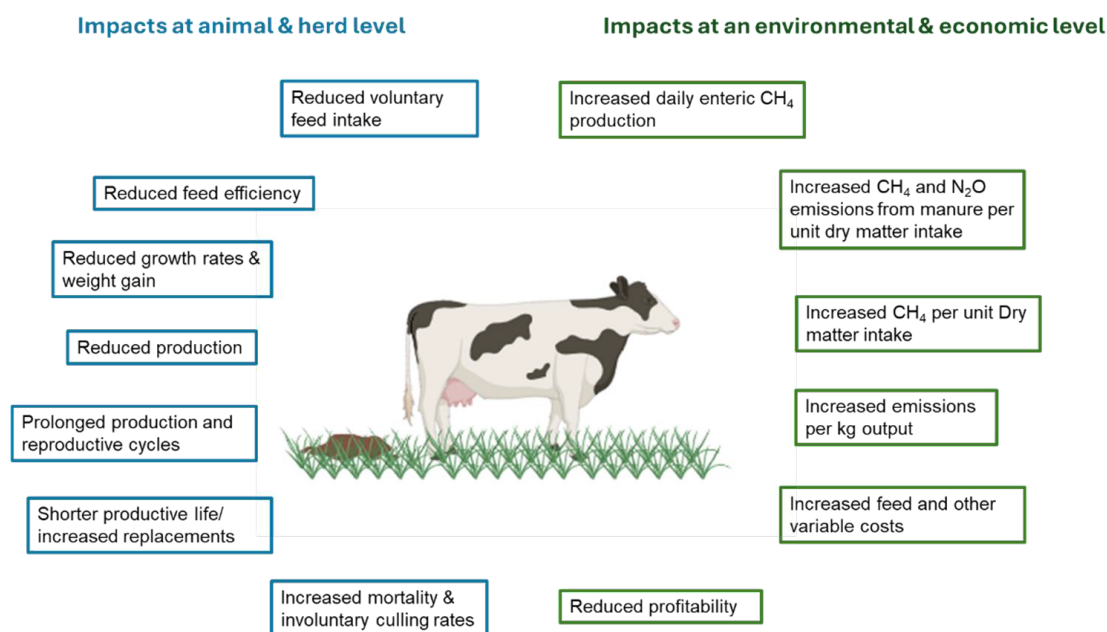


Figure 1. Impact of animal health conditions at animal and herd levels and potential environmental and economic impacts. (Figure adapted from Özkan et al. (2022) and generated using Biorender.com).

Parasitic infections and methane emissions

Gastrointestinal parasitism in sheep reduces feed conversion efficiency and delays market readiness, resulting in a 33% increase in CH₄ emissions per kilogram of dry matter intake (Fox et al., 2018). In parasitised ewes an 11%, 32% and 30% increase in enteric CH₄, manure CH₄ and manure N₂O emissions, respectively per kg lamb weight gain was observed (Houdijk et al. 2017). This was mainly due to increased feed intake of the flock with delayed weaning of lambs from affected ewes and compensation for maternal body condition loss. In naturally infected growing lambs, a 10% decrease in EI was also calculated from the use of suppressive anthelmintic treatments (Kenyon et al, 2013). Similarly, liver fluke infections in cattle exacerbate CH₄ emissions by extending growth periods and lowering weight gains, with studies estimating a 1.5% rise in CH₄ emissions associated with affected animals (Johnson et al., 2022).

Mastitis and emissions in dairy systems

Mastitis reduces milk yields, extends calving intervals, increases culling rates, and can result in discarded milk all of which raise EI. In the UK, Stott et al. (2010) estimated that reducing mastitis prevalence could lower GHG EI by 8% (Stott et al, 2010). In Spanish dairy herds, a mastitis prevention programme achieved a 2.5% reduction in GHG EI by improving milk yield and reducing resource wastage (Hospido & Sonesson, 2005). A potential reduction in GHG EI of 4% was also reported by Özkan Gülzari et al. (2018) in Norwegian dairy herds with subclinical mastitis and a somatic cell count (**SCC**) of 50,000 cells/mL compared to a SCC level of 800,000 cells/mL. Similarly using a stochastic simulation model, Mostert et al. (2019) calculated a 6% increase in EI from cows with clinical mastitis.

Reproductive disorders and fertility impacts

Reproductive disorders illustrate the broader productivity and emissions consequences of poor health. Infections with bovine viral diarrhoea virus (**BVDV**) reduces fertility, prolongs calving

intervals, and increases calf mortality, leading to higher EI. Eradication of BVDV in Northern Ireland improved milk production by 2% and reduced CH₄ emissions in beef herds by 1.5% (Guelbenzu & Graham, 2013). In Scotland, it has been estimated that BVDV eradication could have a significant effect on productivity and reduce CH₄ emissions by 8% per calf sold in beef suckler herds (Stott et al., 2010).

Regional perspectives and challenges

Sub-Saharan Africa

The impacts of livestock disease on GHG emissions are thought to be particularly pronounced in LMICs, where disease prevalence is high, and veterinary resources are limited. Despite this, there have been relatively few studies which have quantified the impact of improved animal health on GHG emissions across the region.

One of the first major studies to investigate livestock disease on emissions in sub-Saharan Africa (**SSA**) modelled the potential impact of Trypanosomiasis eradication in cattle on the EI of milk and meat production (MacLeod et al., 2018). While recognising the different livestock systems across SSA (pastoral, agro-pastoral and mixed farming) as well as the diversity in indigenous and cross-bred cattle breeds, the study demonstrated a 0-8% decrease in EI depending on the system with the highest levels of impact seen in high yielding dairy systems.

By investigating retrospectively at beef and dairy systems, the GHG emissions costs and food loss from calf mortality and abortion have been calculated in Kenyan beef and Tanzanian dairy systems, respectively (Gurmu et al., 2024). Reducing calf mortality rate from 16% to 0% for the first year of life has the potential to significantly reduce emissions intensity of meat within the system by up to 6% while providing additional protein for up to 4.5 million Kenyans at current levels of intake. Likewise, by addressing abortion rates, the estimated EI of cattle and goats would be mitigated by 5% and 16%, respectively, and provide a 1,230 metric tons of milk protein and 1,137 metric tons of meat protein a year; sufficient to provide animal derived protein for nearly 650,000 Tanzanians, based upon current levels of average consumption. While complete resolution of these conditions is unlikely to ever be achieved, the figures demonstrate that even moderate reduction of losses due to these conditions will make a marked impact on GHG EI and productivity.

South America

Similar dynamics are present in South American livestock systems. However, a major bottleneck remains the lack of region-specific modelling efforts quantifying the emissions impacts of key diseases such as tick-borne infections, neosporosis, toxoplasmosis, and ectoparasitic infestations such as the screwworm. Production losses from these diseases are well-documented, but their translation into emissions metrics has yet to be systematically addressed.

Several vector-borne and ectoparasitic diseases prevalent in South America have significant, though underexplored, implications for GHG EI. Tick-borne diseases such as babesiosis and anaplasmosis, transmitted by *Rhipicephalus sanguineus*, are responsible for substantial production losses across cattle populations in the region. These diseases lead to decreased weight gain, reduced milk production, increased mortality, and higher culling rates, all of which contribute to higher EI. Moreover, their geographical distribution is projected to expand under climate change scenarios, further amplifying their impact on both productivity and emissions (de la Fuente et al., 2020).

Reproductive disorders and calf/lamb mortality represent additional drivers of EI in livestock systems in South America. In Uruguay, for instance, *Neospora caninum* is a leading cause of abortion in dairy cattle, resulting in the estimated loss of approximately 12,510 fetuses/calves and 62 million litres of milk annually (Carrillo Parraguez et al., 2025). This substantial production loss implies not only economic hardship for farmers but also inefficiencies that are likely to elevate GHG EI due to wasted resources and extended production cycles.

India

Disease control is a key priority within Indian livestock policy, as evidenced by the launch of the National Animal Disease Control Programme (**NADCP**) in 2019, which aims to eradicate Foot and Mouth Disease (**FMD**) and Brucellosis through comprehensive vaccination campaigns and animal identification initiatives (Government of India, 2019). From a mitigation perspective, these disease control efforts offer significant co-benefits by improving productivity, reducing mortality and culling rates, and consequently lowering GHG EI. India has one of the highest incidences of FMD globally, with an estimated 3.39% prevalence alongside China (Knight-Jones and Rushton, 2013). Modelling by York et al. (2017) calculated that national-level FMD vaccination efforts could reduce EI of milk alone by between 3.68% and 12.95%, highlighting the climate mitigation potential of integrating animal health policies such as the NADCP into national climate strategies.

Modelling animal health in the context of climate change: Methodological challenges

a) There is consensus that Intergovernmental Panel on Climate Change (**IPCC**) Tier 2 approach or higher should be used in calculating the effects of mitigation interventions on GHG emissions as the Tier 1 approach does not account for animal status or the variations in emissions over time (Chang et al., 2021; Özkan et al., 2022; Wilkes and Van Dijk, 2018). However, the existing models that are based on Tier 2 methodology also exhibit limitations. For example, the link between animal health and the change in energy requirement in presence of a health condition is still a missing link since most of the models assume that the feed requirements of animals are met (e.g. GLEAM in Macleod et al. (2018), HolosNor in Özkan Gülzari et al. (2018), and AgreCalc in Lanzoni et al. (2025)).

b) The functional unit by which GHG emissions are reported is of great importance. Here, the two most commonly referred to units are EI and total (or absolute) emissions over a time dimension. The link between reduced EI and improved productivity is rooted in the “dilution of maintenance” effect. In essence, the energy required for maintenance is considered to be fixed and met regardless of productivity (e.g. milk yield or live-weight gain) level. As productivity increases, the proportion of energy allocated to maintenance is diluted over a greater number of production units, reducing the net energy requirement per unit of product. Applying the same concept to GHG emissions, proportionally more milk and meat relative to the amount of GHG emissions leads to reduced EI (Capper and Williams, 2023). There is significant nuance in expressing EI per kg product or per kg energy/per kg nutrient content. Animal-source products that are rich sources of protein, minerals and vitamins typically have a higher energy density than other foods such as grains or vegetables so may have higher EI per 100 gr, but much lower values per 100 kcal (Drewnowski et al., 2015). The expression can also be based on the nutrient density (i.e. % of recommendation in 100 g of products x number of nutrients) per gram of carbon dioxide equivalent (g **CO₂e**) emissions (e.g. beef with high nutrient density and high GHG emissions) (Werner et al., 2014).

c) Allocation of emissions between edible and non-edible products is an important aspect when reporting the results in modelling. Most studies allocate emissions to edible products such as milk and meat protein, but it is important to remember that livestock play additional roles beyond providing food for many smallholder farmers such as providing fertiliser, acting as draught power and as importantly as a financial asset (Weiler et al., 2014). Whether the products or the nutrients are followed out of system can have a significant influence in the modelling results (Frehner et al., 2020). Models may be able to trace emissions linked to inputs e.g. feed and fertilizer, back to the producing country, and allocate the emissions to the consuming country (e.g. in GLEAM). However, it may be more challenging to allocate the emissions of primary animal products and commodities because large volumes of these products are traded globally. In this case, the emissions associated with the trade can be allocated to the consuming country using bilateral trade statistics (FAO, 2023).

d) Despite the significant mitigation potential of animal health interventions, their integration into national climate strategies remains limited. There is no standard way to calculate the impact of animal health status on GHG emissions, making it a challenging task to incorporate it to national climate policies (Özkan et al., 2022). Of the countries with Nationally Determined Contributions (**NDCs**), only four (Albania, Burundi, the Gambia and Sri Lanka) explicitly mention animal health, and none provide quantified estimates of its impact on emissions. This omission is partly due to the reliance on Tier 1 reporting methodologies, which use fixed emission factors per animal and fail to account for the benefits of health-based interventions (Özkan et al., 2022). Transitioning to Tier 2 and Tier 3 approaches which incorporate system-specific data and dynamic modelling, is essential for capturing the full mitigation potential of animal health measures (Wilkes & Van Dijk, 2018).

In the path to incorporating animal health into NDCs, below steps can be useful for countries to prepare, implement, and develop (Özkan et al., 2022):

- Identifying pre-conditions e.g. awareness, capacity building, coordination with other sectors, collaboration with management unit in MRV process
- Defining the role of enabling environment: Responsible for MRV system, institutional arrangement; data collected via regular and systematic animal censuses, monitoring systems, consultations; data system maintained
- Establishing the required baseline of GHG emissions based on Tier 2 approach (or higher): Need to record data on activity, production, losses & waste, feed, and manure are provided by veterinary & animal health services, farm monitoring and disease surveillance, industry partners, and national project management units
- Assessing the expected impacts of animal health condition on activity data: by research and extension, and industry partners
- Calculating the change in emissions and regular monitoring: by the agency responsible for MRV system

Aligning animal health initiatives with climate finance opportunities can significantly accelerate progress. Conditional targets in NDCs are often linked to external funding, and countries with well-defined strategies that include health interventions are better positioned to attract such support. As livestock currently only receive 1% of the climate finance in the agriculture and land use sectors (Buto et al., 2021), remaining one of the least-financed sectors, projects funded by International Financial Institutions are of great importance in increasing the climate finance in the livestock sector (Mottet et al., 2024). Özkan et al. (2024) highlighted how the

DiMMA programme in Georgia leveraged investments in veterinary infrastructure to achieve both productivity gains and GHG emissions reductions, strengthening its case for climate finance. Expanding access to similar funding mechanisms in LMICs could enable the scaling of health interventions while addressing broader development goals. For instance, initiatives that combine climate-smart agriculture with animal health measures could further amplify co-benefits (FAO, 2023). By explicitly incorporating animal health into climate action frameworks, LMICs can unlock new opportunities for investment, leveraging global commitments to both climate mitigation and sustainable development. These efforts can pave the way for transformative change, ensuring that animal health interventions are not only effective in their immediate contexts but also scalable and sustainable over the long term.

Conclusion

Animal health offers a critical yet underutilised opportunity to mitigate GHG emissions, enhance livestock welfare and productivity, and achieve broader sustainability goals. Health interventions, including vaccination programmes and improved veterinary services for disease prevention, treatment, or control, reduce EI while delivering co-benefits for food security, public health, and rural livelihoods. Dynamic modelling approaches and enhanced data collection are essential for accurately quantifying these impacts and informing evidence-based policy. Furthermore, integrating animal health into NDCs and leveraging climate finance can unlock the resources needed to scale interventions globally. By prioritising animal health, policymakers and stakeholders can advance sustainable agriculture and contribute to global climate targets.

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497 Breeding sustainable livestock - global approaches using genomic and metagenomic selection tools to reduce enteric methane

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Abstract

Ruminant livestock supply approximately half of all protein derived from livestock converting pasture into high quality protein (FAO, 2022). They are an important source of meat, milk and fibre. A by-product of this production, however, is the eructation of methane gas during the microbial fermentation of feed in the fore stomach or rumen of ruminant livestock. Globally methane emitted from this pathway is a major source of greenhouse gas emissions, with ~30% of global methane emissions attributed to ruminant livestock (FAO, 2022). International agreements such as the Paris Agreement, the Global Methane Pledge, and alliances such as Race to Zero, engage countries to constrain temperature rise by mitigating greenhouse gas emissions.

Genetic analysis of methane emissions – heritability, genomic selection

Enteric emissions can be altered at the individual animal and system level by factors that affect ruminant digestion. The four main ruminant methane mitigation research areas are: altering genetics of the host ruminant (breeding), altering digestion and microbial composition by using modified feedstuff, altering microbial composition by small molecule inhibitors, and altering microbial composition and function by vaccines.

To date, breeding is the only permanent effective mitigation strategy. Methane traits include:

- Methane production: Total methane output.
- Methane intensity: Methane per unit of output, e.g. milk kg
- Methane yield: Methane per unit of feed intake.
- Residual Methane Emissions (**RME**): Methane adjusted for production traits

Studies spanning over a decade, have consistently underscored that ruminant methane emissions have a host genetic component with differences between individuals in a population shown to be heritable or passed on to the next generation.

Estimates of heritability range from 0.10 to 0.29 for methane production (g/day) and 0.10 to 0.21 for methane yield (g CH₄/kg DMI), depending on species, measurement technique, and data structure (de Haas et al., 2011; Jonker et al., 2018; Pinares-Patiño et al., 2013; Robinson and Oddy, 2016). This means that targeted breeding could result in generations of ruminants with naturally lower methane emissions. Breeding schemes centre around a few main principles. Accurate measurements of the trait being improved or selected for; the use of information from multiple sources such as the breeding candidate, their progeny, and records from relatives; and dissemination of selected germplasm. Traditional breeding schemes

involved breeding many individuals and measuring their offspring or progeny to select the best performers. This can be a lengthy and expensive process with traits such as methane that are difficult to measure. The advent of genomics in livestock breeding schemes and of sophisticated statistical algorithms has led to the ability to accurately predict traits in animals at birth by measuring a representative subset. Implementation involves training data requiring many thousands of records. The number needed is directly related to accuracy of measurement, genetic variation available to select upon (heritability), and effective population size (Hayes et al., 2009). National breeding schemes for lowered methane emissions have their own specific set of challenges. The main challenge is accurate measurement of the trait in a sufficient number of animals at pasture.

For genomic prediction of methane traits, given the genomic structure of ruminant livestock industries, a base training population of ~5-10,000 individuals will be required for prediction of sufficient accuracy. The training population accuracy also needs to be maintained by replacement of around 5-10% new animals per year (Hayes et al., 2009).

Measuring methane for the purpose of using genetics as a mitigation strategy

The main complexity around monitoring enteric methane, is that the gas is generally present as a mixture with other gases and in constant flux. Technical factors for combining data include sampling method, the sensitivity of measuring device, the volume of air, proximity to animal, air flow, temperature, pressure, and animal or biological factors such as age, stage of growth, feed intake, time from start of digestion, volume of feed in the gut, and quality of feed. This makes it difficult to compare technical, temporal, or biological replicates. Respiration chambers can be used to obtain accurate estimates of methane emissions from ruminant livestock; however, costs are prohibitive, throughput low, and results are affected by isolating the animal in a false environment for prolonged periods. To adapt to the requirement for thousands of measures, researchers have developed alternative measurement technologies. These are less accurate ways of recording and predicting methane emissions that overcome the lack of accuracy by enabling large scale testing, thus lowering error rates by harnessing volume either through measuring more animals or more measurements per animal or both.

Sampling of methane using feed stations with enclosed hoods such as Greenfeed (C-Lock Inc., Rapid City, SD, USA) (Zimmerman et al., 2011), by use of tracer gas such as Sulphur hexafluoride (**SF₆**) (Johnson et al., 1994), by gas concentration in sealed units such as Portable Accumulation Chambers (**PAC**) (Goopy et al., 2011) and methane detection devices or spot samplers collectively and colloquially known as 'sniffers' have all been shown to be heritable (Garnsworthy et al., 2012; Goopy et al., 2016; van Breukelen et al., 2024). Limitations and assumptions on the measurement technique must be known and accounted for.

There are a variety of smart devices being designed to monitor animals in real time to provide efficient management of data. These devices may be attached via an ear-tag or nose-ring, temporarily via a collar or headcollar or within the animal itself such as a rumen sensor, however, this field of research is in its infancy, and the utility of wearables for prediction of methane emissions is yet to be validated. Hand-held laser methane detectors, although promising, have also yet to show significant heritable variation due to the environmental variance associated with very noisy data (Chagunda, 2013; Reintke et al., 2020).

Metagenomic prediction

An alternative approach to measuring methane directly would be to obtain an indirect measure of methane emissions via a proxy trait, for the purpose of ranking animals on their methane

emissions. The most promising proxy for methane is the use of rumen microbiota to predict methane emissions, whereby predictions from the microbial data forms a proxy methane trait used to rank animals for methane related traits. This makes biological sense because in ruminants, methane is a by-product of a complex microbial fermentation process in the rumen and therefore changes in microbial profiles are likely associated with differences in enteric methane production in ruminants. Profiles are typically generated by sequencing microbial DNA from digesta sampled from the animals' rumen. This sequence data is used to infer abundance of microbes at the individual genera or species level. These collections are referred to rumen microbial communities (**RMC**). To date, studies have provided evidence that RMC profiles are both heritable and highly predictive of methane in sheep and cattle in multiple studies (Bilton et al., 2024; Difford et al., 2018; Gonzalez-Recio et al., 2018; Huws et al., 2018; Li et al., 2018; Ross et al., 2013; Wallace et al., 2019).

Rumen microbial communities consist of complex, high dimensional data. Incorporation of information from the RMC profiles requires an appropriate statistical model to use this RMC information. To predict methane or other traits of interest, Ross et al. 2013 proposed the metagenome relationship matrix (**MRM**) to describe the relationships between RMC profiles between individuals and used this matrix in a conventional GBLUP type model where the genomic relationship matrix is replaced by the MRM to predict various traits of interest in humans and cattle from the RMC profiles. The term 'microbiability' was introduced by Difford et al. (2018) to describe the proportion of genetic variance explained by the microbial matrix as compared to heritability which describes the genetic variance explained by the animal's genetics. Several studies have used this approach on various species for a variety of traits, where different ways to construct the MRM have been developed (Martínez-Álvaro et al., 2022; Roehe et al., 2016; Ross et al., 2013; Hess et al., 2020; Wallace et al., 2019).

A few studies have estimated the genetic correlation between some form of RMC methane trait with methane traits measured using various technologies with genetic correlations estimates moderate to high. Identifying the microbes present to estimate abundance matrices is limited to availability of suitable reference microbial genome sequences. Isolating and sequencing the obligate anaerobes is challenging and a large proportion remain unidentified. Hess et al. (2020) used a restriction enzyme reduced sequencing approach and circumvented this barrier by binning sequence data by similarity metrics in a reference free approach. This improved power and accuracy considerably by improving information content. The advent of concerted sequencing projects such as culturomics (Waters et al., 2025), MAG sequencing and improved databases such as the Genome Taxonomy database (**GTDB**) (Chaumeil et al., 2020) mean reference-free and reference-based approaches are now closer in accuracy. Reference-based approaches have the advantage of also being biologically informative as well as predictive. The general consensus is that predictive models that combine both host and microbial genetic information have the greatest accuracy. Bilton et al. (2024) determined the genetic and phenotypic parameters of RMC proxy traits for methane with methane measured from PAC for sheep using a dataset consisting of over 4500 animals by fitting bivariate models to estimate the genetic and phenotypic correlations of the RMC proxy traits with the PAC methane traits. Christensen et al. (2021) proposed a joint model that accounts for genetic effects mediated by intermediate traits, such as RNA, which directly link DNA action to phenotype expression. While Christensen's work focused on RNA as an intermediate trait, the underlying principle – using intermediate layers to capture genetic effects – can be extended to other omics data, including the rumen microbiome. Zhao et al. (2022) expanded the methodology of Christensen

et al. (2021) by introducing the Neural Network-Mixed Model (**NN-MM**), a Bayesian framework allowing for different heritabilities for each omics feature. This method might be an optimal solution for integrating rumen microbiome data. While the rumen microbiome is not a direct intermediate trait of the host (RNA is an intermediate trait between DNA and phenotype), it is directly involved in enteric methane production and fitting it as an intermediate layer in methane trait prediction is cogent (Boggio, 2023). As such, although RMC data are not strictly intermediate traits, they serve as a source of information between genotype and phenotype. Alemu et al. (2025) showed that incorporating RNA sequence data from host tissues improved accuracy of genomic prediction by 10%.

Statistical standardisation and scaling of microbial sequence data is complex and an area that is currently under discussion. Some approaches such as the cohort standardisation approach used by Hess et al. (2020), show adjusting for environmental effects improves accuracy of prediction and provides a good option for ranking selection candidates. These methods, however, do not maintain microbial dimensionality with critics arguing that centred log ratio (**CLR**) approaches are more appropriate. Other scaling methods akin to the standardisation of genomic relationship matrices by population frequencies are also under development.

As statistical methods are developed that incorporate microbiomes into prediction models, the mass capture of samples has become more pertinent. Limitations to sampling rumen fluid are that it is an invasive method as the rumen fluid is collected using an oesophageal sampling device. Additionally, a lack of standardisation across animal ethics committees makes the consistency in collection difficult as opinions on the optimal restraint procedures and sampling equipment vary from country to country. Rumen microbiomes are dynamic and affected by sample handling and storage, so it is important that standardised protocols are used for statistical comparisons. An alternative to rumen sampling which has increased animal ethical acceptable is the use of swabs to collect fluid from the buccal cavity. Whilst this approach is showing promise, it has yet to be demonstrated as having sufficient efficacy to replace rumen sampling as a means of estimating the RMC of the animal.

Sequencing approaches range in breadth and depth from amplicon approaches and marker genes through restriction enzyme approaches such as that used by Hess et al. (2020) that capture 1% of the microbiome compared to full metagenomic sequencing.

Country-level implementation examples

To date, national schemes are limited to implementation of genomic prediction. Several countries have made significant strides in incorporating methane traits into their breeding programs. In New Zealand, a national evaluation is in place with methane breeding values available for sheep using PAC data and genomic selection (Rowe et al., 2019). Evaluation schemes using PAC in sheep are also under development in Australia, Ireland, UK, Norway and France. In Australia, the Sheep CRC and Meat & Livestock Australia have invested in genetic and genomic evaluations, including methane yield and residual methane traits. In Denmark, methane is being recorded via sniffer systems in commercial dairy herds, and genomic evaluations are being first published in 2025 (Schneider et al., 2025). In the Netherlands, methane is recorded large-scale in commercial dairy farms with sniffers (van Breukelen et al., 2024). A breeding value for methane production has been available since April 2025 (de Jong, 2025). In Ireland, GreenFeed systems are used to record methane emissions in dairy and beef cattle and the Irish Cattle Breeding Federation published the first enteric methane across breed evaluations on artificial insemination (**AI**) sires with phenotyped

commercial progeny in 2023 (www.icbf.com). In Canada, Semex have incorporated MIR-predicted methane into the national dairy evaluation. Genomic selection models are being developed using GreenFeed phenotypes and milk-based proxies (van Staaveren et al., 2024). In Uruguay national evaluations for beef and sheep methane traits are being disseminated and in New Zealand LIC and CRV are selecting dairy bulls for divergent methane and evaluating their daughters.

Current international research

There are large national and international programs developing knowledge and technology in order to harness new technologies in existing breeding schemes.

In Spain, between 2018 and 2024, methane emissions were recorded in more than 3000 cows in 34 commercial farms using sniffers. Currently, routine methane measurements are being taken on farms with automatic milking systems as part of the national recording scheme organized by CONAFE. Recently a new project named D1-HOLI is recording methane in 12 additional farms (50% organic) using GreenFeeds. Microbiome samples are obtained from 15-20% of cows in the projects.

The Resilient Dairy Genome Project (**RDGP**) is led by the University of Guelph, Canada. Objectives of the RDGP are to develop genomic tools to improve the resilience of dairy cows, enabling them to adapt to changing environmental conditions while maintaining productivity, health, and fertility, and reducing their environmental impact. The RDGP project combines fundamental research, industry collaboration, and student training to translate genomics into practical applications. Activities include DNA sequencing and methylation analysis, development of national pipelines for fertility, disease resistance, and environmental efficiency traits, and integration of GE3LS research to align breeding objectives with farmer priorities and societal acceptance footprint (van Staaveren et al., 2024). Data management, modelling, and genomic prediction workflows were established to ensure the practical use of research outputs. The project produced multiple genomic tools implemented by the dairy company Lactanet (<http://www.resilientdairy.ca>).

In Ireland, through a number of international projects such as RumenPredict and EU Horizon 'MASTER', the rumen microbiome has been examined in a population of over 1000 Irish beef cattle with performance and methane emissions data. A relationship was identified between the rumen microbiota and Residual Methane Emissions (**RME**). Animals ranked as low RME had the highest relative abundances ($P < 0.05$) of lactic-acid-producing bacteria and *Selenomonas*, and the lowest ($P < 0.05$) proportions of *Pseudobutyrvibrio*, *Butyrvibrio*, and *Mogibacterium*. An increased abundance ($P < 0.05$) of the genus *Methanosphaera* and *Methanobrevibacter* RO clade was observed in low RME animals. The relative abundances of both *Intestinibaculum* and *Olsenella* were negatively correlated ($P < 0.05$) with RME and positively correlated with ruminal propionate (Smith et al., 2022; Waters et al., 2025). More recently, the collection of rumen samples has been extended through a number of projects such as Methane-Predict (IE-NZ collaboration) and MAGS (DAFM funded) and contributed to the EU Horizon HoloRuminant Project (with partners from Spain, Ireland, France, Italy, Finland and Canada), which aims to establish rumen microbial guilds associated with methane emissions and the investigation of novel genetic markers associated with these guilds that could be incorporated as microbiome assisted GEBVs to improve the selection of cattle with low methane emissions.

The International Livestock Research Institute (**ILRI**), Nairobi, Kenya, in collaboration with the Scottish Rural College (**SRUC**) are focused on development of breeding schemes for lowered environmental impact and increased productivity in Sub Saharan Africa (**SSA**). Given the increasing trends in climate change in terms of increasing temperatures, research into the sustainability of the smallholder system has become of paramount importance. A project on reducing feed costs and GHG emission in smallholder dairy cattle in SSA (briefly refereed as Environ-Cow) was initiated in Ethiopia and Tanzania in 2023.

In New Zealand, there is a national evaluation delivering genomic breeding values for daily methane production to sheep breeders. This is supported by Beef and Lamb New Zealand, AgResearch and the New Zealand government through the Cool Sheep program. The evaluation is well established and has been running for over 5 years. Over 7000 methane measures are collected each year using trailed portable units that travel across the country. Research breeding values are also being produced by AgResearch that provide breeding values for methane production generated from genomic and microbial information. This program is being extended to Beef under the 'Cool Beef Program' aided by the development of portable accumulation chambers for cattle. LIC and CRV have been measuring dairy bulls and their daughters and have shown genetic variation amongst dairy bulls for methane emissions. A new program evaluating the relative efficacy of the prediction of methane breeding values from genomics, rumen microbial profiles, oral swabs and milk profiling has just started run by AgResearch and funded by the Ag Emissions centre.

A new initiative, the Global Methane Genetics Initiative (Gredler-Grandl et al., 2024), coordinated by Wageningen University & Research with funding from the Global Methane Hub and the Bezos Earth Fund, has been put in place to accelerate genetic progress in methane mitigation in ruminants. It is designed to enable genomic selection for reduced enteric methane (**CH₄**) emissions in ruminants. The program involves large-scale phenotyping (>110,000 cattle and sheep) using standardized CH₄ measurement protocols, in combination with genotyping, pedigree recording, and rumen microbiome characterization (~20,000 samples) across 25 countries and 25 breeds. Data will be stored in a centralized global database to support international genetic evaluations. Standard operating procedures for CH₄ measurement and quality control are being developed in collaboration with the ICAR Feed & Gas working group to ensure interoperability across measurement systems. The resulting reference populations will facilitate estimation of breeding values for CH₄ traits and enable integration into routine national and international genetic improvement programs.

Concluding remarks

Our recommendations for livestock industries are that where feasible, direct measures of methane are most effective for calibration, validation and for measuring influential animals such as dairy bulls. Methods have been developed that are cost effective for sheep and goats. Current infrastructure, however, is insufficient for the measuring of tens of thousands of animals to build the training populations required for genomic prediction in cattle and deer. A rapid scaling of measures is needed. For population-based analyses, rumen microbial profiling is straight forward and can be carried out at a relatively low cost in hundreds to thousands of animals. Proxies for developing countries that require a small amount of infrastructure together with the wrap around support required for breeding design, phenotype collection and ranking of animals could make a major change to greenhouse gas emissions both directly by breeding for lower enteric methane emissions but also indirectly by selecting for greater efficiency. Global collaborations including Global Research Alliance livestock research group (GRA,

2025) GRA flagship programs and the support of the GMH will accelerate international collaborative research and facilitate greater measurement of methane across country and system, and better access to technology and validation which will be key to driving progress in reducing enteric methane from ruminants.

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483 Management practices to increase circularity at farm level by reducing nutrient losses from manure

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Abstract

Livestock manure is a critical source of plant nutrients and organic matter, ensuring high crop productivity and continued soil health and providing a source of income for farmers. At the same time, the intensification of livestock production has led to increased manure production, often exceeding the nutrient requirements of the land available for its application. In addition, decoupling animals and crops for intensive production systems has led to challenges for nutrient recycling. This imbalance results in nutrient losses, particularly nitrogen (**N**) and phosphorus (**P**), leading to environmental pollution through increased greenhouse gas (**GHG**) and ammonia (**NH₃**) emissions, runoff, and leaching. This not only exacerbates global warming and soil, air and water pollution but also constitutes substantial nutrient losses from the agricultural system. This keynote presentation explores the potential of circular animal manure management to reduce nutrient losses and mitigate emissions, runoff, and leaching by increasing nutrient recycling at the farm level, focusing on both intensive and extensive livestock systems across different geographical contexts.

Strategies to reduce nutrient losses can be implemented at different stages of the manure management chain, i.e. animal housing, manure storage, manure processing, manure spreading, and grazing management (Chadwick et al., 2011; Petersen et al., 2013). The selected strategies will depend on the level of intensification, availability of equipment, financial resources and labour, and the environmental and climatic context. In addition, socio-cultural aspects, farmer training, and adoption of best management practices are important. These vary greatly among the Global North and Global South and between production systems, creating a need to develop targeted interventions that are context-specific and feasible for the farmer. Below is a description of several examples from different regions, describing local contexts, priorities, and suggestions for circular manure management practices.

Intensive (crop-) livestock systems

Intensive dairy and pig systems in Europe are often zero-grazing, with animals remaining indoors 24/7. Housing typically consists of closed barns with concrete flooring and slurry-based manure management, with slurry (mix of dung and urine) flushed out of the barn using water, followed by collection in tanks, ponds and lagoons. These slurry stores constitute an anaerobic ecosystem, resulting in methane (**CH₄**) emission hotspots (Kupper et al., 2020). One intervention to reduce **CH₄** from slurry is frequent emptying and cleaning of the slurry inside animal housing and the lagoons or tanks outside. This lowers levels of viable methanogenic microorganisms and reduces **CH₄** emissions from housing by 50-90% (Dalby et al., 2023) and from slurry storage by 40% (Haeussermann et al., 2006). Another option to reduce **CH₄** emissions is to cover slurry with porous materials, such as straw, clay pebbles, or recycled polyethylene, as this may increase **CH₄** oxidation (Sommer et al., 2000). Similarly, slurry

aeration reduces CH₄ emissions by 50-100% through introduction of O₂ (Amon et al., 2006; Martinez et al., 2003), but this practice leads to substantial emissions of nitrous oxide (N₂O) and NH₃ (Loyon et al., 2007), resulting in up to 50% of N being lost from the slurry (Molodovskaya et al., 2008). Acidification of slurry can reduce NH₃, N₂O and CH₄ emissions during storage (Berg et al., 2006), and also decreases emissions after manure application (Petersen, 2018). This concept is being further developed in novel technologies such as plasma-treatment of liquid manure (Nyang'au et al., 2024).

A common way to make use of the high CH₄ emissions from slurry is through anaerobic digesters (**ADs**), where manure is decomposed under anoxic conditions and the produced CH₄ is collected for the use as biogas to generate power. The resulting digestate or “bioslurry” is a valuable source of plant-available nutrients as the ammonium (NH₄⁺) content is increased (Möller, 2015; Möller and Müller, 2012) and the digestion process also solubilises P (Li et al., 2020). GHG balances for manure and biowaste management using ADs were carried out for five Danish biogas scenarios in which pig and cattle slurry were co-digested with organic waste and plant residues (Møller et al., 2022). All scenarios significantly reduced GHG emissions, mostly from fossil fuel substitution and reduced CH₄ emissions. On the other hand, ADs increase pH and NH₄⁺ concentrations in the manure, which increases the potential for NH₃ emissions if no counter-measures (e.g. covering or acidification of the digestate, soil injection instead of surface application) are taken.

In the Global South, liquid manure management is increasing as farming systems become more intensive. In Vietnam, where the climate is hot and humid, slurry storage is common in pig farming because animals are hosed down with water twice a day to cool them, as pigs cannot sweat and might overheat. The slurry is either directly discharged into the environment, collected in slurry tanks, or used in small-scale ADs (Thien Thu et al., 2012). Similarly, larger-scale pig producers and slaughterhouses in Uganda often handle liquid manure, which is either stored in uncovered lagoons or discharged into the environment (Wanyama et al., 2021). This has substantial implications not only for farm-level nutrient losses, which are large if manure is not recycled on crop fields but allowed to flow freely into rivers and soils, but also for animal and human health, as pig manure carries several pathogens of animal and zoonotic diseases and gastrointestinal parasites (Dione et al., 2014; Roesel et al., 2017). If slurry is being collected and used as fertilizer, slurry stores should be covered or acidified to prevent NH₃ volatilization and retain more N in the system (Misselbrook et al., 2016).

Small-scale ADs are being promoted across sub-Saharan Africa (**SSA**) and South-East Asia (**SEA**) as a solution to manage liquid manure and produce biogas as a clean energy source. In addition, in countries such as Brazil and Chile, ADs are becoming increasingly important for pig manure management. For dairy cattle systems, this technology is not common due to the low potential for CH₄ production from dairy slurry, high costs of AD plants, and low organic matter content of dairy slurries originating from grassland systems (Salazar et al., 2010).

The AD technology has several advantages that are beneficial for developing countries. For example, avoiding deforestation by reduced need for firewood (Subedi et al., 2014), lower expenditure for energy (Kelebe, 2018), and better household air quality, which particularly benefits women and small children (Pizarro-Loaiza et al., 2021). Also, the reduction of GHG emissions is important for national inventories and international country commitments such as Nationally Determined Contributions (**NDC**).

However, challenges still exist when it comes to efficient management of small-scale digesters, for example if too much water is mixed into the slurry, which reduces its retention time and with that, biogas yield (Thien Thu et al., 2012), or if ADs are leaky and release large amounts of CH₄ into the atmosphere (Bruun et al., 2014). In addition, many smallholders lack knowledge and capacity to handle liquid bioslurry, which often leads to it being discharged into the environment (Kelebe, 2018; Thien Thu et al., 2012), creating an N and P loss pathway from the farm and a source of environmental pollution. Furthermore, there is often a lack of local service providers that can help farmers during construction, operation and maintenance of ADs.

A way to overcome this is drying or composting of bioslurry to facilitate handling, but this results in high NH₃ and N₂O emissions and reduces the N content of the remaining organic fertilizer (Salamat et al., 2022). Therefore, efforts to increase the efficient use of small-scale ADs should not only focus on process optimization for better biogas production but also ensure that farmers are equipped with the knowledge and tools to safely handle, store, transport, and apply liquid bioslurry to ensure nutrients are recycled within the farm.

In most zero-grazing or “cut-and-carry” systems of SEA and SSA, however, manure is managed as solid, often mixed with bedding materials such as straw, sawdust, and feed refusals (Ndambi et al., 2019). In smallholder dairy systems of the Kenyan highlands, farm sizes are small, with 2-5 cattle and 0.5-2 ha of cropland (Frelat et al., 2016). Manure is used as organic fertilizer and is often the only source of plant nutrients (Sheahan and Barrett, 2017). However, manure management in these systems is usually sub-optimal, leading to high GHG emissions and N losses and exposing animals and humans to pathogens through contaminated drinking water, flies and other vectors, and direct contact (Surchat et al., 2023; Wanyama et al., 2021). The collected solid manure is often stored in heaps without cover, exposed to sun and rain, which reduces manure N content by up to 75% (Tittonell et al., 2010). The poor fertilizer quality of the manure that ultimately reaches the cropland is exacerbated by low-N diets of animals that are fed on carbon-rich, N-poor feeds such as maize stover, overgrown Napier grass, or low-quality hay, culminating in feed shortages during dry seasons (Goopy et al., 2018; Ndung'u et al., 2018). Manure with a very low N content cannot meet crop N requirements, which, together with high costs of synthetic fertilizer, contributes to the continuously high yield gaps in SSA, compromising food and nutritional security of millions of people (Leitner et al., 2020; Mueller et al., 2012).

Manure management interventions should therefore address, increased nutrient recycling, climate change mitigation, and animal and human health simultaneously to develop recommendations that are feasible for smallholder farmers and ensure that the managed manure is a good-quality and sanitary organic fertilizer. Composting is a promising manure management option for smallholders addressing health risks (Couth and Trois, 2012). The aim should be to increase compost temperature >50 °C to kill pathogens and weeds, and the compost heap should be mixed to ensure that all material is heated (Bernal et al., 2009). Challenges of composting are additional labour requirements (Oberlin and Szántó, 2011; Surchat et al., 2023) and high N losses in the form of NH₃ and N₂O emissions (Chadwick et al., 2011). However, when facing the trade-off between health and nutrient recycling, the authors think that the safety and health of animals and humans must come first, and that manure sanitization should be ranked higher than nutrient circularity or reduction of emissions. As a consequence of health hazards, pig manure is often discharged into the environment in some regions in Uganda (Wanyama et al., 2021), instead of being sanitised and used to recycle

plant nutrients within the farm. Moreover, co-composting with biochar could increase composting temperature, reduce pathogens and weeds, and reduce N losses and emissions during composting (Agyarko-Mintah et al., 2017; Chung et al., 2021). In Kenya and Uganda, small-scale artisanal biochar production using waste products (e.g. maize shanks, rice husks, coconut shells, wood from invasive trees) is being taken up by smallholders who value the benefits of biochar on soil health and crop yields (Basalirwa et al., 2024; Kätterer et al., 2019; Roobroeck et al., 2019). Currently, biochar is usually applied directly to the soil, not making use of its potential to increase nutrient retention and composting efficiency during manure storage. Further studies should explore using biochar in the manure management chain, potentially starting in animal barns by adding a layer of biochar on top of the bedding material, followed by composting of the manure-bedding-biochar mixture, up to cropland application, to improve nutrient circularity in smallholder systems.

Extensive livestock systems

In extensive systems, livestock spend most of their time grazing in rangelands, with varying levels of time spent in confinement, ranging from a few hours per day to <1 month per year. In the Global North, such grazing systems can be found in the UK, Ireland, and New Zealand in the form of zero-housing, all-year-grazing sheep or beef production systems where animals may only be housed for lambing/calving (Barthram et al., 2002; Sagar et al., 2015). Similarly, in extensive dairy production systems in the Austrian and Italian Alps, cattle stay outside from spring to fall and are only housed during winter months (Latruffe et al., 2023; Sturaro et al., 2013). In these extensive systems, excreta from grazing animals are usually not managed but faeces and urine are voided directly on the pasture. Here, urine patches are the main source of excreta N₂O and NH₃ emissions (Chadwick et al., 2018; Van der Weerden et al., 2020). Mitigation strategies such as optimizing animal diets, utilizing silvopastoral systems, incorporating N-fixing plants, and use of biological nitrification inhibitors (BNI) can lower emissions while simultaneously enhancing soil quality and C capture, potentially achieving reductions of up to 50% in emissions (Gerber et al., 2013; Rivera and Chará, 2021). Additionally, including tannins at low levels can shift nitrogen excretion from urine to faeces, thereby minimizing N₂O emissions (Gerber et al., 2013).

A special case integrating extensive and intensive livestock production is the production of beef in feedlots in Argentina, Brazil, USA, and Australia where animals are first raised extensively on grasslands and are then placed in feedlots where they receive energy- and protein-rich diets based on grain and concentrates for fattening. Feedlots are sources of high NH₃, CH₄ and N₂O emissions and N leaching (Aguilar et al., 2014; Laing et al., 2023; Waldrup et al., 2016), losses that can be reduced through frequent manure removal. Collected manure is often stored in heaps, sometimes with composting, and then either used on crop fields or sold (Costa Junior et al., 2013; DeLuca and DeLuca, 1997). These heaps should be stored on water-proof surfaces to reduce leaching, and runoff from rainwater should be collected in retention ponds and recycled for agricultural use. Specialized feedlot producers who do not have croplands face the challenge of managing large amounts of animal manure. In such cases, composting to reduce volume and odour followed by selling of compost to neighbours or fertilizer producers, as well as biogas production for heat and electricity might be suitable manure management options.

In the Global South, extensive systems in the form of pastoralism, agropastoralism, or silvopastoralism are important sources of livelihood and food. In Kenya, around 80% of beef is produced in pastoral systems in semi-arid rangelands, where livestock keeping is the main

form of agricultural production because these areas are too dry for crop production (MacCarthy, 2000). Here, animals graze on savanna grasslands during the day and are taken to rivers or water pans for watering once a day. Overnight, animals are confined in fenced corrals (“bomas” in Kiswahili) to protect them from theft and predators such as lions and hyenas. They are typically of round shape, with a diameter of 15-30 m (area 200-700 m²), and house around 50-150 animals (Butterbach-Bahl et al., 2020; Leitner et al., 2024; Zhu et al., 2024). These bomas can be temporary, used only for a few days to months as herders migrate with their satellite herds, or they can be permanent if they are located at the homestead, where they house adult females with young calves or sick animals (Lamprey and Reid, 2004). Manure is usually not removed from bomas, and N deposition rates in can be 7000 kg N ha⁻¹ or higher (Leitner et al., 2024), creating excessive N loads compared to plant and microbial demand. In fact, manure loads in bomas are phytotoxic so that they remain bare of vegetation for years after abandonment, and vegetation only regrows after most of the boma-N has been lost (Veblen, 2013; Vrieling et al., 2022). This makes bomas hotspots for N₂O (Butterbach-Bahl et al., 2020; Leitner et al., 2024) and NH₃ emissions and NO₃⁻ leaching (Carbonell et al., 2021). Nevertheless, the pastoralist tradition of confining livestock in bomas creates nutrient-rich hotspots for livestock and wildlife and influences plant species distribution for decades (Muchiru et al., 2009; Veblen, 2013; Veblen, 2012), which has shaped East African savannas over the past three millennia (Marshall et al., 2018). Given their relevance to pastoralists, the aim should not be to eradicate bomas, but efforts should be taken to develop practices that increase nutrient recycling back to the soil, for example via short-rotation bomas or by using boma manure as a fertilizer for land restoration and feed/food production (Veblen and Porensky, 2019). The practice of using nutrients “harvested” in grasslands and transferring them to croplands is also common in West Africa. In the Sahel, grazing animals are confined overnight on croplands before planting, often as a service provided by pastoral communities to crop farmers, creating a net nutrient transfer from grassland to cropland (Schlecht and Buerkert, 2004; Powell and Turner, 1996). From a nutrient cycling perspective, such systems can work as long as there is enough grazing land available compared to cropland and as long as livestock densities are adjusted to avoid overexploitation of nutrients in grazing lands and to prevent grassland degradation.

For South American countries, most of the dairy production is based in grazing systems with reduced housing (Herrero et al., 2018). However, there has been a recent intensification of dairy production with increasing use of concentrates and off-farm feeds and more housing. In these systems, manure management is a key issue where nutrient recycling and reduction of cleaning water are important aspects to be improved on farms (Herrero et al., 2015; Salazar et al., 2010).

Extensive livestock systems are sometimes criticised for having high GHG emission intensities (emissions per litre of milk or meat), followed by a call for “sustainable” intensification (Jones et al., 2014; O’Brien et al., 2016). However, from a nutrient cycling perspective, grazing systems are ideal because the nutrients (in manure) are deposited at the site where they were first consumed (as feed), and soil nutrient stocks are continuously replenished through animal inputs, creating a circular system. Nevertheless, nutrient losses still occur in the form of emissions, runoff, and leaching as well as landscape-level translocation between grazing areas, where nutrients are removed, and watering or confinement areas, where nutrients are concentrated, and through migratory or transhumant movement of animals between winter/summer or dry season/wet season grazing grounds. Nevertheless, in addition to being

(almost) circular, extensive livestock systems have several other benefits, ranging from increased animal welfare (Von Borell and Sørensen, 2004; Windsor, 2021), grassland conservation and biodiversity protection (Battaglini et al., 2014), to cultural heritage preservation (Smith, 2019).

Conclusions

Circular manure management practices offer significant potential for reducing GHG and NH₃ emissions, runoff, and nutrient leaching at the farm and landscape level. By closing nutrient loops and enhancing nutrient use efficiency, these practices contribute to more sustainable and climate-resilient agricultural systems. The adoption of circular manure management practices varies across different farming systems and regions. In intensive systems, technological interventions such as composting, AD, and nutrient recovery processes are more prevalent, while extensive systems may rely more on natural processes and traditional practices. However, the principles of nutrient recycling and GHG mitigation are applicable across all systems. The effectiveness of circular practices is influenced by factors such as farm size, infrastructure, and access to technology. Policy support, farmer incentives, and extension services play crucial roles in promoting the adoption of these practices, particularly in smallholder and resource-constrained settings. Tailored approaches that consider specific contexts and challenges of different farming systems are essential for maximizing the benefits of circular manure management. In addition, implemented mitigation practices should not cause pollution-swapping; therefore, best-management practices should consider integrated studies to optimise animal-soil interactions, increase nutrient efficiencies, and reduce losses to the wider environment.

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482 Opportunities for data based on-farm estimation of greenhouse gas emissions from ruminants farming systems

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Abstract

Livestock farming has become increasingly digitized in large parts of the world. Digitally most advanced farms have invested in technology mainly to increase productivity and improve economic outcomes. In recent years also technology developed mainly for sustainability monitoring purposes or software to use existing systems for sustainability reporting have been introduced. The data that farmers collect to monitor and optimize their production offers a possibility to increase the accuracy of greenhouse gas (**GHG**) emission measurement if utilized fully. However, cost benefit and reliability of farm level data collection needs to be considered when deciding on priorities in developing national inventories and Life Cycle Assessment (**LCA**) models.

Currently lack of access to data and poor interoperability of the data across different software systems is a broadly recognized issue. Clearly, if the data cannot be accessed it cannot be utilized. In addition to accessing the data it needs to be understood by the users. Standardization of measurement methods and data formats is important for the efficient utilization of data. Even if the data is accessible in a standardized format the quality of the data cannot be guaranteed. Poor calibration of equipment such as animal and feed weighing equipment can lead to significant errors in the collected data. Quality management schemes are needed if this type of equipment is to be used for GHG accounting purposes.

Equipment based on more complicated calibrations or models such as spectral analysers, wearable sensors measuring feeding behaviour or computer vision-based methods can suffer from poor accuracy if they are used in environments which are not covered in their training datasets. These types of systems typically cannot be calibrated on the farm and their performance on novel environments should be validated. Moreover their (mis)use for other purposes, like prediction of different traits (intake, emission, metabolism) assessed by correlation, may lead to strong biases in decision outcomes.

The aim of this presentation is to assess the state-of-the-art of the digital data collection usable for GHG accounting from ruminant farming systems from an on-farm perspective. The majority of emissions from ruminant systems originated from enteric methane, manure management, and feed production and we aim to cover data sources related to each category. The focus will be on assessing the value of existing data collection systems, possibilities of accessing and combining data from heterogeneous systems and measures needed to increase the reliability of the on-farm data collection.

On-farm digital data collection

This section reviews the potential of digital technologies and widely kept on-farm records for collecting data on animal productivity, feed properties, feed production and manure management. Precise data based management of farming systems is commonly referred to

as precision agriculture, a management strategy that gathers, processes and analyses temporal, spatial and individual plant and animal data and combines it with other information to support management decisions according to estimated variability (ISPA, 2025). The term precision livestock farming is also commonly used when referring to livestock systems. Due to their origin in precise management of resources, precision agricultural technologies collect detailed animal-level data and high resolution spatial data of field management. A lot of on-farm data can also be collected manually and recorded using e.g. mobile phone applications. For GHG accounting purposes manually recorded observations can be equally valuable as long as those can be accessed in digital format.

Animal productivity

The milk production of animals in intensive systems is recorded in great detail. With automatic milking systems, the yield of each visit is recorded and stored in a database. Several cow, sheep, and goat milking parlours also include yield monitoring technology and collect the daily milk yield data. Solutions for smallholders include mobile phone applications where the milk production of individual animals can be recorded and monitored. The weight and the weight gain of the animals have been historically recorded using weighing scales, and also relatively affordable systems for small ruminants have been developed. Walk-over-weighing scales that digitize data have been adopted by production systems with extensive areas of grasslands. These can also be linked to decision-support tools and make automatic changes in grazing systems, such as the 3-way auto drafting. Computer vision-based systems for animal weight, body condition score, and rumen volume estimation have been studied and are being introduced into the market, at least for cattle, and these can be based either on fixed cameras or images taken by the user with a mobile phone.

Feed intake and feed composition

Feed intake measurement on research farms is commonly done with weighed feeding bins, which are too expensive and laborious for farmers use. Feed composition can be recorded in the feeding system by the farmers. For total mixed ration (**TMR**), most accurate systems guide the preparation of the planned ration with scale and the weight of each component is recorded. In addition, the properties of each feed component should be known. The components used for feed and the quality of forage can vary significantly throughout the year and frequent analysis of the feed would be required to ensure high-quality recording.

Currently, inventories and LCA models estimate the feed intake using prediction models based on animal milk yield, weight and feed properties. Recently, several technical solutions have been developed for more direct measurement. With the help of advances in 3D imaging and deep learning, a commercial system for measuring individual cow feed intake called CFIT (Viking Genetics, Denmark) has been developed and commercialized. The system is based on estimating the volume of feed which each animal eats at a feeding visit and high agreement (mean absolute error 4.89%) with the weighted feed was achieved in a validation trial (Giagnoni et al. 2024). The system has already been used to phenotype over 6000 cows from commercial farms in Denmark (Manzanilla-Pech et al. 2023). To estimate dry matter (**DM**) intake from the consumed feed volume the density of the feed needs to be known.

Another option for estimating on farm use of DM intake is the use of accelerometer tags worn on the neck or ear tags of the animals. These accuracy of several systems for measuring grazing, feeding or rumination time has been validated for high or medium accuracy (Stygar et al. 2021). The technology is applicable to all ruminant species, although commercial systems

mostly focus on cattle. The use of feeding time to predict DM intake is not very accurate as the feeding rate of individual animals can vary and can also be influenced e.g. by the properties of the feed.

The highest uncertainty of the amount and quality of animals' intake could probably be in extensive and heterogeneous grasslands, in which animals graze freely and selection intensity is high. Some behaviour studies have indicated differences when estimating enteric methane emissions from animals with diverse grazing behaviour (Ricci et al., 2014).

Feed cultivation

Emissions originating from forage and cereal production can vary greatly between farms within a region or within the same farm across growing seasons mainly due to differences in fertilizer use and yield variability. Farmers often keep detailed records on the used inputs and also harvested yield, however the yield is often estimated and not measured.

From high technology farms, the emissions from feed production could at least in theory be accounted for at sub parcel level. The data is recorded by the equipment but might not necessarily be stored or accessible for the farmers. The yield of forage and cereal production is commonly measured using yield monitors. More recently NIR sensors have been added to forage harvesters to measure dry matter and protein content. The primary use of yield monitors is to provide an accurate map of spatial variability in the field to identify different management zones and their absolute accuracy is limited (Schneider et al. 2024). This indicates that the more laborious option of weighing each trailer and analysing the dry matter content from samples still provides more accurate data

For pasture based systems ground based biomass measurement methods include rapid pasture meter C-Dax which can be attached to an all-terrain vehicle driving in the field, rising plate meters and the use of remote sensing for biomass estimation. All of these methods have significant prediction error (e.g. Ortega et al. 2022, Ogunbuyi et al. 2024).

Sharing of on-farm data

Data sharing is the buzzword used to describe various forms of access to data from different software and sensor systems. It is a common misconception that data sharing refers to free access to data, however agricultural data can hold significant value for the farmers and they should not be forced to share business critical information unnecessarily to public authorities, food companies or retailers without fair compensation.

In Europe the data sharing within and across sectors is envisioned to be done with Common European Data Spaces which are seen as an essential component in the European strategy for data (EU Commission, 2020). The strategy has the ambitious aim of making the EU a role model for a society empowered by data. First commercial data intermediary services making sharing of data with consent management made easy for farmers are now operational. These services are built on the "enter data only once" principle aiming to eliminate duplicate data entry needs.

The principle of data sovereignty, central to the vision of seamless data exchange, is defined as *"Data sovereignty ensures that organizations, governments, and individuals have control over their data. This control allows them to self-determine how their data is collected, stored, shared, and used by others. Data sovereignty is a spectrum; it's about finding the right balance between keeping data safe and sharing it to gain added value."* by the international data spaces association (IDSA).

European data spaces are based on two key specifications, the IDSA reference architecture model (**RAM**) and Gaia-X for decentralized identity. The vision for data spaces is to have decentralized soft infrastructure that is based on mechanisms for identity and consent management and include mechanisms for data monetisation. From a high level perspective the data ecosystem can be seen as a collection of interoperable APIs (Nagel and Lycklama et al., 2021). Data sharing in the data spaces is based on legally enforceable agreements binding data space participants as well as transaction participants. Many other potential technological solutions for the same problem could be developed, for instance Romera et al. (2024) proposed the use of Web3.0 technologies for facilitating efficient flow of livestock data in New Zealand.

Probably the most advanced data space currently is the Catena-X automotive network which is based on the open source Tractus-X project. Several concepts from the project could also be used in agricultural context. All assets are represented by digital twins (**DT**) holding the data related to e.g. individual car parts, these digital twins each have unique identifiers and consistent semantics and a standardized API. Each DT can cover the whole lifecycle of an asset or there can be several DTs related to different stages of the life cycle. This unified representation of assets allows building services on top of the data such as standardized and automatic product footprint calculation. The same concept could be applied to collecting livestock data where each animal, each device etc. could be represented as a digital twin with agreed upon data sources and semantics. Establishing such joint representations has obvious benefits, however significant efforts are needed to develop digital twins or other similar constructs representative of different production systems.

Use of data to assess methane emissions

Direct on-farm measurement of methane is currently only feasible with specialized equipment. Assessment of on-farm methane emissions is thus mainly based on prediction models fitted to experimental datasets. The most important predictor for methane emissions is the dry matter intake (**DMI**) of the animal and additional accuracy can be achieved by including diet characteristics into the model. As DMI cannot be easily measured on farms it is usually predicted based on estimated energy requirement and diet composition (Vibart et al. 2021; Hristov et al. 2018). The DMI prediction accuracy varies across farming systems and regions where prediction of herbage intake in pasture-based systems has the greatest uncertainty. Consequently, it is needed to account for those variabilities in the calculation, and sensitivity analyses are a useful methodology to achieve this goal (Blondiaux et al., 2024).

Obtaining accurate diet composition data would therefore be the key parameter which is currently not broadly available, this data could be obtained by combining feeding system data and laboratory analysis data. Further, the farmers need to be able to obtain representative samples from their feed and be willing to have each feed component analysed.

If daily records of feeding from TMR feeders can be used, the emission factor for methane could be calculated daily using an approach developed by Bannink et al. (2020) where tabulated diet specific emission factors were developed for the Netherlands using a mechanistic model, or another option is to rely on empirical modelling (Eugène et al., 2019).

Use of data for LCA

Development of high-quality livestock farm-level LCA datasets is crucial for high quality estimation of the environmental performance of livestock products, independently of the selected system boundaries and functional unit. On a dairy cattle farm, complete farm-level

LCA datasets would include qualitative and quantitative information about the product outflows, the land use and land use change for the farm, all the product inflows such as feed components and / or compound feed mixes', bedding materials', and air (e.g. methane, nitrous oxide, ammonia, etc.) emissions' outflows, manure outflow and other solid waste outflows. This information is typically completed in a time boundary of one year of farm's operation. Further relevant material inputs for feed crop production need to be considered, such as synthetic and organic fertilizers and pesticides as well as outputs such as water (e.g. nitrate and phosphate ions, etc.) and soil (e.g. pesticide chemicals) emissions.

Due to the importance of the cattle farm operations in the environmental performance of the product outputs (e.g. FAO, 2016), primary farm-level data is necessary for completing the farm-level LCA datasets. Apart from the invoices for all material inputs purchased, the water and electricity meter and cattle population data (for all cattle sub-categories housed on-farm), primary data needs for completing the farm-level datasets are dependent on the emissions' estimation approach. For GHG emissions, on-farm data availability usually allows for employing IPCC Tier 2 approaches (e.g. Gavrilova et al., 2019 or national inventory approach) or higher Tier to estimate cattle-based emissions such as enteric methane, methane and direct nitrous oxide from manure management, and indirect nitrous oxide due to ammonia and nitrogen oxides. Cattle sub-category body live-weight and live weight gain, cattle sub-category on-farm population, feed intake and characteristics (e.g. DM, GE, DE, CP etc.), the type of manure management system and the relevant emission factors are necessary parameters for estimating the latter emissions.

Use of calibrated and valid data from digital data collection technologies could contribute to enhancing the quality of the dairy cattle farms' LCA datasets, as it ensures high technological, temporal and geographical representativeness of several inputs and outputs. It further ensures that estimation of model uncertainty, implying an increase in the total data quality score of the dataset (Zampori and Pant, 2019).

Complementary to the enhancement of the farm-level LCA dataset quality, exploitation of this digital data could allow for differentiation between the lowest and highest emitting farms in a region, with the prerequisite that the LCA dataset development follows a standardized procedure for all dairy cattle farming systems in this region. Farm specific LCA data collection has been introduced by dairy companies in multiple countries e.g. by Fonterra in New Zealand (Fonterra, 2025).

Discussion

The use of on-farm data for GHG accounting provides an opportunity for more targeted mitigation and even differential product pricing favouring the lowest emitting farms within a region. Farm specific LCA using Tier 3 approaches is already a reality in many countries, we estimate that a lot of the data is still manually entered into the systems and sensor data is not commonly used. The greatest potential for automating the LCA process and coverage of production for national inventories exists for dairy cattle, sheep and goat farms with daily milk yield recording and high technology feeding systems and lowest for pasture based meat production systems.

The reliability as well as contribution to overall emissions of each additional data source collected from farms should be assessed before it is taken into use. Calibration protocols for farmers to ensure accurate data should be developed and their application monitored. Rigorous regional validation is needed for black-box technologies which can't be directly

calibrated, such as machine learning models running on embedded systems. In addition, if sensor data is used for GHG accounting, collaboration is required with sensor companies to ensure that products undergo new validation after significant software or hardware updates.

Digital tools can and likely will play an increasing role in the future, we need to ensure that the technologies which will be used are reliable and cost efficient. A lot of the on-farm, in particular from sensor systems, cannot currently be easily accessed. That is, however, expected to change with the introduction of data spaces in Europe and other similar initiatives around the world. Economic incentives through payments from consumers or subsidies should be considered to accelerate the adoption of broader sustainability monitoring technologies which do not increase the profitability of farms.

Farmers can implement emission reduction measures, or at least believe that they have, which are not captured in the national inventories. For example, in Finland the Tier 3 LCA emission method available to dairy producers can use more data sources than the national GHG inventory, the reason is that farmers self-report the data for LCA for the dairy company but is not collected by public records. This can lead to disparity of views on the farmers participation on climate action between the farms, policy makers and the general public. The true participation of farms can be either over or underestimated unless the impact of local activities can be captured in the inventory. It is paramount that public support can be directed to actions which are measurably effective which requires detailed collection of activity data.

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493 Scaling-up livestock-based mitigation and improving GHG inventories - The perspective of an international development finance institution

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The case for investing in livestock for climate action

Livestock are a game changer for the rural poor. They provide food and money to the majority of the 700 million people who live in extreme poverty, playing a critical role in incomes, food security and nutrition. Worldwide, about 750 million people suffer from hunger and the diets of an estimated two billion people are deficient in micronutrients, especially in iron, calcium, zinc, vitamin A, B12 and folate (FAO et al., 2023).

Animal products are essential for closing the nutritional gap of the rural poor, because they provide higher-quality protein than other foods and are denser in several micronutrients, in more bioavailable forms. Livestock also provide fuel and fibre as well as a form of savings. They are a common asset that can be used for direct cash needs and can serve as insurance for times of crisis, for example during a drought, a flood or a crop failure. Livestock increase crop productivity through manure that is used as fertilizer and through traction for working land and transporting people and goods. Livestock are essential parts of agroecosystems and they also serve nonmarketable roles in the livelihoods of the rural poor, including important cultural value. About 60 per cent of rural households keep livestock in a wide variety of production systems, with a range of 40 to 93 per cent of households in sub-Saharan Africa (Kaur et al., 2017). Smallholder livestock keepers and pastoralists, however, are among the most vulnerable to climate change. Climate change affects livestock directly, for example through heat stress or lack of water, and indirectly, for example through reduced fodder quality and quantity. Livestock are also globally identified as significant contributors to greenhouse gas emissions, including methane through enteric fermentation, and indirectly through feed and fodder production.

Decision-makers in governments and financing institutions may, therefore, be under the impression that a good climate strategy would be to reduce or stop investments in livestock. This is likely to be detrimental to the livelihoods, food security and nutrition of hundreds of millions of smallholders and pastoralists (Figure 1) and may also result in higher emissions in the medium term if the sector's development is left without public interventions to identify and promote climate co-benefits (Mottet et al., 2024; IFAD, 2024).

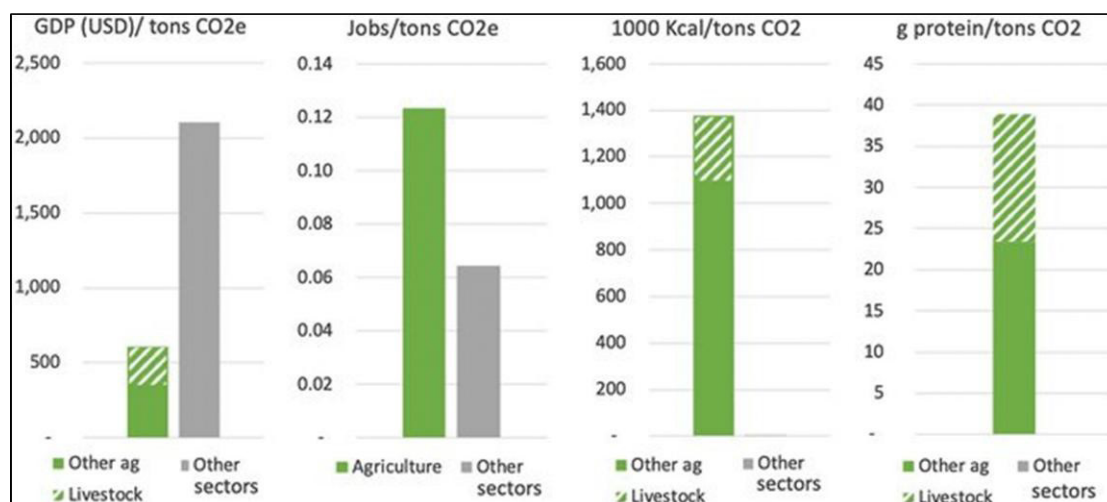


Figure 1. What do we get from a ton of CO₂? (from Mottet et al., 2024).

Mitigation potential in LIC and LMIC in livestock value chains

The potential for reducing greenhouse gas (**GHG**) emissions from livestock through improved practices is high. Adoption of existing best practices to increase productivity could help the global livestock sector cut its GHG emissions by 29 per cent in 2050 compared to 2015 and by 20 per cent compared to a business-as-usual (**BAU**) 2050 scenario (FAO, 2023). Further improvements in breeding, feeding, animal health and manure management can lead to an additional 32% of emission reduction compared to BAU 2050. Solutions are available for all systems and regions (Mottet et al., 2016), which means that improvements in livestock production that also aim at achieving climate outcomes (known as “climate-smart livestock production”) need to be tailored to each system and value chain and each country context. This should not be detrimental to food security and nutrition.

Methane emissions represent a higher share of total life-cycle emissions for livestock in low-income countries (**LICs**) and low- and middle income countries (**LMICs**) (72 per cent and 56 per cent respectively) than in high-income and upper-middle income countries (38 per cent). This indicates that livestock GHG emissions in LICs and LMICs are caused less by producing and transporting feeds, inputs and post-farm emissions (processing, transport and retail). The focus for mitigation in LICs and LMICs should, therefore, be on primary production on farm, to reduce direct emissions in absolute terms and per kg of products, with a specific attention to methane.

However, only about a third of all countries include livestock in their Nationally Determined Contributions (**NDCs**), and only 18 per cent and 16 per cent include specific measures such as manure or feed management respectively as mitigation options. This shows that, despite the potential of livestock for climate change mitigation, governments still need support to improve the involvement of the sector as a solution for their climate commitments.

The high share of methane emissions in LICs and LMICs also signals a strategic opportunity: targeted investments in on-farm productivity, feed efficiency, and animal health can yield disproportionately large mitigation benefits per dollar spent. These interventions not only reduce emissions but also strengthen food security, rural incomes, and resilience to climate shocks, which are key development priorities in these regions. However, realizing this potential will require stronger policy support, tailored technical assistance, and financing mechanisms that prioritize both climate outcomes and inclusive rural development.

Scaling up mitigation actions in livestock value chains

While pilots exist at modest scale to test mitigation options in livestock, large-scale implementation can be achieved through coordinated efforts across the value chains, such as the public-private low-carbon dairy initiative in France, with over 20,000 farms already engaged (<https://www.ferme-laitiere-bas-carbone.fr/decouvrir-le-projet/>). Similarly, the **CARAT** initiative in Pennsylvania (Climate-smart, Profitable, Regenerative, Actionable, and Trustworthy) illustrates a successful collaborative model. By bringing together the private sector, public institutions, and the scientific community, CARAT enhances the competitiveness of the dairy livestock sub-sector while delivering meaningful mitigation outcomes (<https://agsci.psu.edu/research/collaborative/carat>). This can also be achieved by large private companies of the meat and dairy sectors, such as the Net Zero initiative of JBS (<https://jbsfoodsgroup.com/our-purpose/net-zero>) the Brazilian multinational company and largest meat processor in the world. Santos et al. (2022) provided a review of the challenges and opportunities of carbon neutrality in agrifood systems and included animal protein as a key sector in their analysis. They showed that an increasing number of companies are including carbon neutrality in their corporate strategies by working directly with supply chain actors to reduce emissions. They also conclude that smallholder farmers, who represent the largest number of producers and account for about a third of the world's food, remain generally excluded from such opportunity due to lack of access, organisation and training while operating in highly fragmented supply chains.

Their inclusion is, however, easier, and sometimes guaranteed, in the context of public development projects, such as those led by International Financing Institutions. Scaling up mitigation actions in livestock value chains requires a participatory approach that includes smallholder farmers and local communities. Empowering these groups through capacity-building initiatives, training, and knowledge-sharing will enable them to adopt climate-smart practices effectively. By integrating these efforts into community-based solutions, mitigation strategies can be tailored to local contexts, enhancing both environmental and socio-economic outcomes.

The International Fund for Agricultural Development (**IFAD**) specifically targets smallholders and has a corporate commitment to achieve 45% of climate finance in its 2025-2027 portfolio, and is currently at 40%. IFAD's investments in livestock target primarily small-scale production and pastoralism. From a portfolio of 415 projects approved by IFAD since 2010, 65% included livestock investments. The number of projects specifically focusing on the livestock sector was 102 (or 25%) for a total budget of USD 6.2 billion (IFAD, 2025). These projects aim at improving productivity, resilience, and market access for smallholders and pastoralists and they are distributed across all regions, with greater budgets and a number of projects in the Near East North Africa region, West and Central Africa and East and Southern Africa. In Latin America and the Caribbean, IFAD investments in livestock are less specific and are found as activities included in projects focusing on value chain development, natural resources management, or rural finance.

Most of the projects supporting livestock development target improved productivity of livestock and natural resources as well as the provision of essential services to support market systems. Animal husbandry (including improvements in breeding and feed) and rangelands/pasture management (including reducing land degradation) are the two most important areas of investment in IFAD's livestock specific portfolio, followed by livestock advisory services (Figure 2). Animal-restocking projects are mainly targeted towards the provision of productive assets

to vulnerable households. Animal health investments focus on the delivery of the “last mile” services to improve the access of smallholders and pastoralists to such services.

The average IFAD project that includes livestock investments amounts to about USD 85 million and reaches about 800,000 beneficiaries.

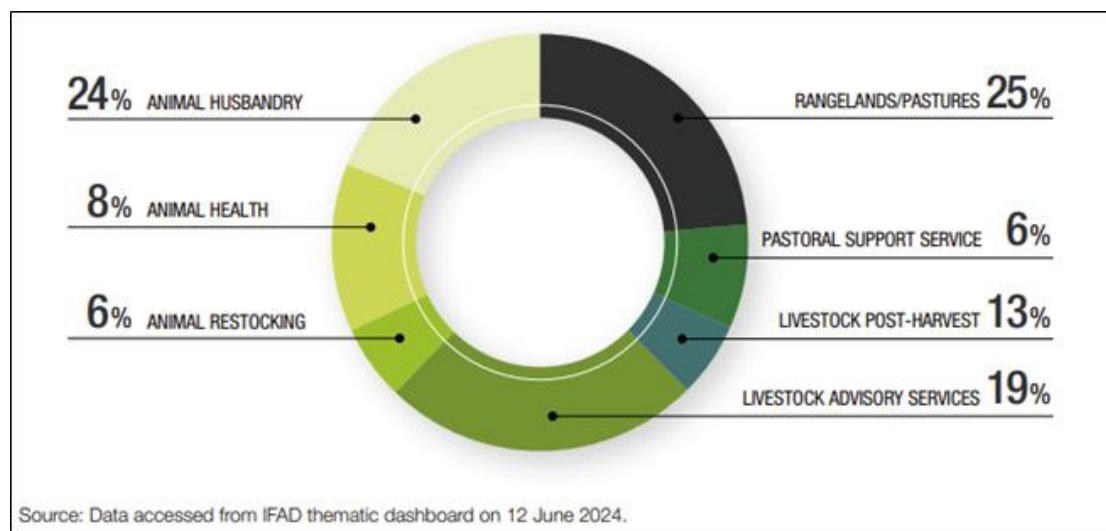


Figure 2. Share of IFAD investments since 2010 per area of livestock development (sample: projects targeting livestock specifically).

Mainstreaming climate-smart livestock interventions in development projects

IFAD uses 5 entry points through which the fund includes climate change mitigation in its investments in livestock: animal health; animal genetic resources (and management of reproduction); feed/fodder strategy and quality; manure management; pasture/rangeland management and livestock mobility.

1. Animal health (and the One Health Approach): Healthy animals produce more and better-quality products and need less resources for the same output. IFAD finances access to veterinary services and preventive care, especially for the “last mile”, and helps strengthen national and local animal health services. The One Health approach, which considers the health of animals, humans, and the environment as interconnected, is integral to IFAD's strategy. For example, in Kyrgyzstan, IFAD invested more than USD 70 million in the Livestock and Market Development Programme I and II, which have helped 2.6 million people build a stronger pastoral economy, including through capacity-building of private and public veterinary service providers, large-scale vaccination campaigns and provision of 630 motorcycles to access remote rural locations at reduced cost (IFAD, 2022). As a result, the incidence of brucellosis was reduced by 25 times in cattle and by 5.6 times among people.

2. Management of animal genetic resources: Improving animal genetic resources through better breed and reproduction practices can boost productivity and resilience. IFAD projects finance crossbreeding local animals with exotic breeds to retain adaptive traits while increasing productivity. This approach helps reduce the number of unproductive animals, thereby lowering GHG emissions. For instance, in Bolivia, IFAD invested nearly USD 40 million in the Pro-Camélidos project to help more than 90,000 people breed more productive llamas, crossing native breeds with improved ones and reducing inbreeding to help farmers produce more meat

and wool while retaining the resilience of local breeds to local conditions. As a results, the average natality rate increased by 36% and the average live body weights of llamas increased by 6%.

3. Feed and fodder quality: Improving feed quality and digestibility reduces enteric CH₄ emissions. IFAD promotes the use of local feed resources, fodder trees, and alternative fodder crops to enhance feed quality and availability. Feed supplementation with natural and synthetic additives can also reduce methanogenesis in the rumen. For example, in Rwanda, IFAD invested more than USD 70 million in the Rwanda Dairy Development Project, which helped the dairy value chain adopt more climate-smart practices and technologies and benefited more than 700,000 people. This included the cultivation of improved fodder (86% of farmers who received support through Livestock Farmer Field Schools). In addition, crop and legume integration practices improved from 15.7% of beneficiaries when the project started to 43.2% at completion.

4. Manure management: Effective manure management is part of a circular bioeconomy, returning nutrients to the soil and reducing reliance on synthetic fertilizers. IFAD supports practices such as composting, biogas production, and the use of indigenous techniques like zai pits to improve manure management and reduce GHG emissions. For example, IFAD will invest in 6,800 biodigesters for small dairy farms in Kenya, Uganda, Tanzania and Rwanda, as part of a USD 360 million project called Dairy Interventions for Mitigation and Adaptation (**DaIMA**), proposed for co-financing to the GCF and targeting directly 2.5 million rural people.

5. Pasture and rangeland management: Grazing management is crucial for improving productivity, resilience, and carbon sequestration in extensive and pastoral systems. IFAD invests in pasture restoration, improved grazing management and securing access to grazing lands in different seasons to support sustainable rangeland management. In Tajikistan, the IFAD Livestock and Pasture Development Programme supported the creation of 203 pasture user unions (**PUUs**) for almost 24,000 beneficiaries and over 80,000 hectares of pastures with improved management, including restoration activities, rotational grazing, and construction of water points and sheds.

The impacts on GHG emissions and carbon sequestration of these 5 entry points are summarized in Figure 3. They include reductions in emissions from unproductive animals (e.g. healthier animals emit less GHG to produce more output), from keeping less animals for the same output (e.g., less animals kept as replacement herd), from reduced enteric fermentation due to higher feed/fodder digestibility, from reduced methane and nitrous oxide from manure and from increased carbon sequestration in pastures with improved grazing management. Figure 3 also lists the co-benefits of these interventions in productivity and in adaptation are also explained.

ENTRY POINT	IMPACT ON PRODUCTIVITY	IMPACT ON ADAPTATION/ RESILIENCE	IMPACT ON EMISSIONS AND SEQUESTERING CARBON
Animal health	Higher milk yields, fertility rates, growth rates Lower losses of products	Lower vulnerability to heat stress, droughts, diseases and other stressors	Healthier animals, which emit less GHG to produce more output
Animal genetic resources and management of reproduction	Selection of animals based on production traits such as higher yields and weights Improved fertility rates	Keeping traits such as resistance to diseases or heat, adaptation to lower feed quality, etc., through conservation of local breeds	Fewer animals needed for the same output More resilient animals, requiring less inputs Expression of genetic potential for higher productivity, resulting in needing fewer animals for the same output
Feeding and feed quality, including fodder	Higher digestibility Higher fertility Mitigation of seasonal availability of feed and fodder	Use of varieties selected for resistance to heat, drought or flood Use of fodder trees for shade, water conservation and seasonal availability	Higher digestibility resulting in lower enteric CH ₄ emissions and possibly changes in excretion and emissions from manure (higher or lower) Avoided emissions through local feed sourcing Sequestration in fodder and trees
Manure management	Increased soil fertility and food/feed crops' productivity	Lower reliance on synthetic fertilizers Improved soil health, including water retention and organic matter content	Lower CH ₄ and N ₂ O emissions from manure storage and application Lower emissions from producing and transporting synthetic fertilizers
Pasture/rangeland management and animal mobility	Improved grazing management resulting in higher biomass productivity in pastures and rangelands, and better animal nutrition with positive impact on health and fertility	Restored rangelands with higher carrying capacity and less vulnerability to climate shocks	Well-managed pastures and rangelands better able to store carbon High-quality fodder, more digestible and resulting in less enteric CH ₄

Figure 3. Entry points for including climate change mitigation in livestock investments, and their co-benefits for productivity and for adaptation.

Promoting simple indicators and tools to measure climate outcomes from investments

So far, low-emissions (or carbon neutrality) processes in agrifood systems have been voluntary. Only a few private companies rely on third-party independent certification for estimating their emissions and most actors do it in-house, which means they typically set their own standards and methodologies. This approach lacks independent validation, undermining the credibility of climate claims (Santos et al., 2022). In the case of development projects led by international financing institutions, approaches and methodologies tend to be more standardized.

IFAD has recently started to include more systematic *ex ante* climate risks and GHG assessments as part of its Social, Environmental and Climate Assessment Procedures (**SECAP**), and in line with its commitments to report its climate finance volumes using the Multilateral Development Banks (**MDB**) methodology. In the absence of such assessment, a livestock project is more likely to be classified as “significant risk”, triggering additional studies and measures to be conducted before and during implementation. Such assessments of large-scale investments in the livestock sector contribute not only to develop solid MRV systems in countries but also to improve countries' reporting in the GHG inventories when they are

correctly captured. This requires the following conditions to be met, as outlined by Özkan et al. (2022):

1. Tier 2 and higher methodologies are necessary to estimate GHG emissions reductions from improved livestock management (including animal health, feed/fodder, breeding and manure), but simple tools and indicators should be prioritized;
2. A data collection and maintenance system needs to be established with the contribution of a variety of stakeholders;
3. A life cycle assessment perspective needs to be considered to account for the reduction in indirect emissions (e.g., changes in feed consumption, pastures, use of energy);
4. The capacity of governments and project partners needs to be enhanced in tools and data for calculating emissions with Tier 2 methodology;
5. Research and academia partners as well as the private sector (e.g. meat and dairy industry) need to be mobilized for establishing references and standards and for accessing key data such as animal weights, milk yields, feed rations, reproduction performances etc.

IFAD is using the FAO Global Livestock Environmental Assessment Model – *interactive* (**GLEAM-i**) in addition to, or in place of, the EX-ACT tool for GHG accounting. GLEAM-i is used for estimating the impact of IFAD projects and also to build capacity in the countries.

Figure 4 presents the results of GLEAM-i assessments for a sample of IFAD livestock projects. Results show that IFAD projects contribute to reducing GHG emissions, especially methane, compared to a situation without investments. If animal production increases more than productivity, absolute emissions may still increase, even if emission intensities decrease (e.g. LPDP II Tajikistan and RDDP Rwanda). This means that additional measures can be put in place to ensure a decrease in absolute emissions, which has been addressed in subsequent project phases (CASP+ Tajikistan and RDDP2 in Rwanda, which additional financing from GCF). IFAD SECAP indicators could be strengthened with additional specific indicators derived from these assessments, looking at absolute emissions and emission intensities (i.e. per unit of product such as litre of milk and kg of meat).

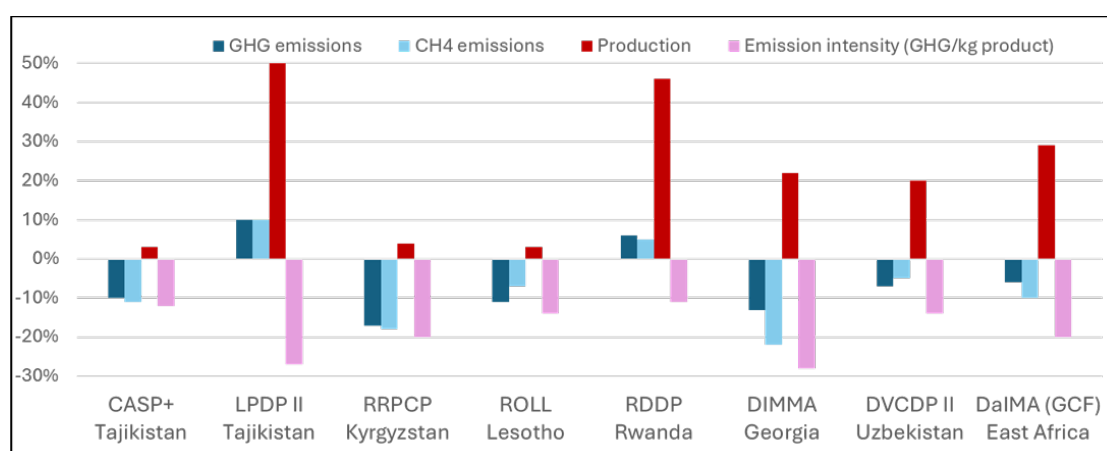


Figure 4. Results of the GLEAM-i analysis of a selection of IFAD livestock projects.

Building capacity in countries

Building capacity for Tier 2 GHG emissions calculation in livestock is essential to ensure that large scale investments in the sector are captured in NDCs and inventories. This includes training project teams in countries as well as government staff (both in agriculture and in environment ministries, as well as statistics services) on livestock-specific tools and methodologies, including data needs. By developing these capacities, IFAD ensures that countries can effectively monitor and report on their GHG emissions, contributing to global climate goals.

To ensure that assessments of large-scale investments are captured in NDC and inventories, results need to be made available to the responsible authorities (see for example GIZ et al., 2021 for Kyrgyzstan). They also need to be aligned with inventory methodologies, meaning that direct and indirect emissions need to be reported separately. Finally, they need to be reported compared to a Business-As-Usual scenario rather than a baseline situation, as most LMIC commit to reduce their emissions (Figure 5).

Effective integration of livestock mitigation data into national inventories also depends on strong coordination between agriculture ministries, environment agencies, national statistics offices, and climate focal points. Establishing institutional linkages and clear data-sharing protocols is essential to ensure that GHG reductions achieved at the project level are reflected in national reporting under the UNFCCC. Without this coordination, valuable emissions data may remain siloed, undermining countries' ability to accurately track progress toward their NDC targets.

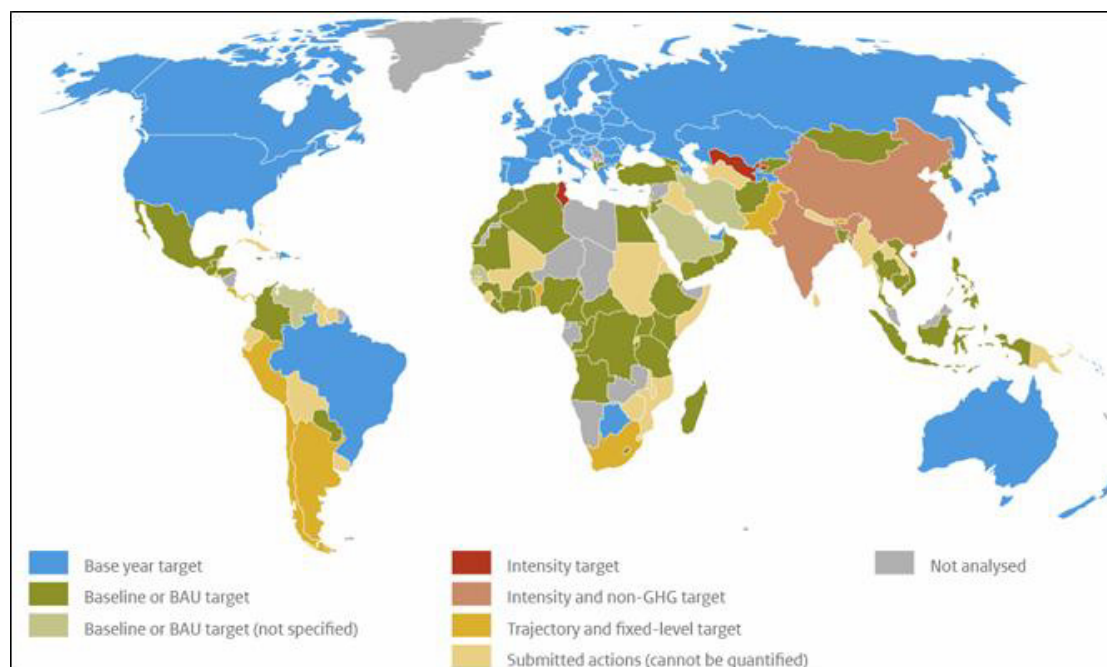


Figure 5. Most LMIC commit to reduce emissions compared to BAU, not to baseline. Source: <https://themasites.pbl.nl/o/climate-ndc-policies-tool>

Improving access to finance to develop low-emission livestock value chains

Financial institutions often perceive small scale agriculture or pastoralism as too risky and are reluctant to lend money to farmers and agribusinesses. Climate change increases the risks

and investment reluctance. Farmers themselves are reluctant to borrow for agricultural production, because of their difficulty in managing risks such as climate related shocks and livestock disease. Individual small-scale producers have small, highly diverse and highly geographically dispersed operations, and assessing their individual risks is costly. For livestock, the absence of a movable asset registry can also be a limitation on the use of animals as collateral. Funding needs to be channelled through aggregators such as project developers and financial institutions, which can increase the funding costs.

Traditionally, farmers and pastoralists have insured their livelihoods through social capital and animal asset building. These surplus animals also emit GHGs. Many IFAD projects increase access to financial services and loans, so that small-scale livestock producers can invest in their businesses and increase their productivity. However, in pastoralist systems, adoption remains very low. Trustworthy insurance, secure saving facilities (including through information and communications technology) and investment options for pastoralists can shift asset capital from livestock to other (monetary) assets, and therefore contribute to reducing GHG emissions. For microcredit, trust can be built through farmer-controlled saving schemes, trusted banks or innovative solutions (e.g. index-based livestock insurance schemes, early warning systems) (IFAD, 2023).

IFAD's main target group – smallholder farmers – only receive 0.8% of climate finance while being among the most vulnerable. Attracting private sector funding and blended finance can help fill this climate financing deficit. GCF, GEF, and Adaptation fund are all financial partners that can contribute to bring no-cost or low-cost monies into blended finance structures. While this is a new area of work for IFAD, two examples are available, in partnership with the GCF: the ARCAFIM project, which provides de-risking to attract public and private investors that would otherwise not invest, and the DaIMA project in East Africa, which will provide financing options for dairy farmers and value chain actors to reduce emissions, including methane.

To better align finance with climate outcomes, investment mechanisms that reward emissions reductions, such as results-based financing, carbon credit schemes, and performance-linked loans, can be deployed alongside traditional grants and subsidies. These tools provide an additional incentive for farmers, cooperatives, and agribusinesses to adopt low-emission practices, especially when integrated with technical assistance and MRV systems. Furthermore, aligning financial products with climate metrics can attract private sector actors seeking to meet sustainability targets, creating new pathways for blended finance and scaling impact.

Supporting public policies and enabling environment

IFAD recognizes the importance of supportive public policies for climate-smart livestock development and uses evidence from its operations to inform the policy dialogue. The fund advocates for regulatory policies, incentivization, pricing/trade adjustments, and research and infrastructure development to create an enabling environment for low-emission livestock production. This is an important part of the DaIMA project in East Africa for example.

Conclusion

Investments in low-emission livestock production should be guided by a commitment to improving rural livelihoods, food security, nutrition and climate resilience. They should also be seen as essential to NDCs and national GHG inventories. By mainstreaming climate-smart livestock interventions, promoting simple indicators and tools, and supporting capacity

development and public policies, IFAD aims to achieve better climate outcomes while continuing to support the livelihoods and nutrition of small-scale livestock producers.

For international development finance institutions, this represents a strategic opportunity: by integrating climate-smart livestock investments into broader rural development efforts, they can simultaneously advance national climate goals, strengthen food systems, and improve livelihoods. Scaling such integrated approaches is essential not only for delivering on NDC commitments but also for achieving long-term resilience and inclusiveness in agrifood systems.

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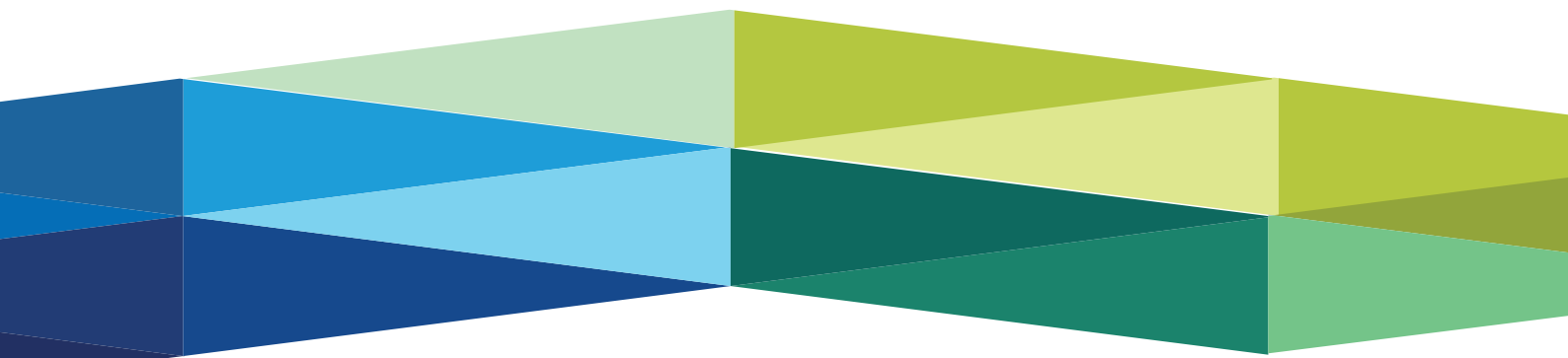
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Abstracts of oral presentations and speed poster presentations

Theme 1: Impact of animal feed and nutrition on greenhouse gas emissions

1.1 Dietary supplementation to reduce ruminant greenhouse gas emissions – Feed additives (part I)



74 Kinetics of bromoform metabolites and bromide in dairy cows fed *Asparagopsis*: single and multi-dose experiments

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Asparagopsis spp. is a potent methane inhibitor in ruminants, due to its high levels of bromoform and other halogenated metabolites. Limited knowledge exists on the kinetics of bromoform and its bromine metabolites in ruminants. *In vitro* studies showed rapid bromoform metabolism into dibromomethane and other bromine metabolites. This study investigated the kinetics of bromoform and its metabolites in rumen fluid, blood, faeces, urine, and milk in dairy cows. Experiment 1 involved 4 rumen fistulated lactating dairy cows housed in a tie stall barn (16 days adaptation, 3 days sampling), fed a 35% grass silage, 22% maize silage, and 43% concentrate diet (DM basis), and on d 17 received a single dose of 150 g freeze dried *Asparagopsis* (2570 mg of bromoform) via the rumen fistula. Samples were taken from feed, water, and animals at baseline (0h) and from blood, urine, faeces, and rumen fluid at 1, 2, 4, 6, 8, 12, 24, 36, and 48h post-dosing. Milk samples were collected at 3, 6, 9, 12, 24, 36, and 48h post-dosing by complete milking. Noncompartmental analyses were performed to calculate pharmacokinetic parameters, and averages are presented. Experiment 2 involved 12 intact lactating dairy cows randomly assigned to one of three treatments in a block design. Cows were housed in a tie stall barn (14 days adaptation, 21 days treatment administration), and fed a 52% grass silage, 25% maize silage, and 23% concentrate diet (DM basis). Cows received daily treatments at 0500 and 1700 h with a drench gun containing 200 mL water plus 25 g dextrose, or 200 mL water plus 25 g dextrose with either 12 g freeze dried *Asparagopsis* or 32 g oil steeped with *Asparagopsis* (0, 384 and 384 mg bromoform/d, respectively). Samples were collected daily (urine and faeces, 0700h; milk, 0800h) or bidaily (blood, 0700h) and analysed for bromoform, dibromomethane, bromomethane, dibromochloromethane, arsenic, bromine and iodine. Results of this experiment are in progress and will be presented at the conference. Preliminary results from experiment 1 indicated no bromoform or its metabolites were detected in feed, drinking water, or animal samples at 0h. In blood, urine, and milk, bromoform and dibromomethane reached peak concentrations (16.5 to 82.5 and 2.0 to 77.0 ng/mL, respectively) between 2 and 6h after dosing, falling below detection limit by 24 to 36h. Bromoform and dibromomethane were not detected in faeces. Bromine was detected in feed (2.1 to 44.5 mg/kg) and in animal samples at 0h (2.9 to 10.2 mg/L), but not in drinking water. Peak bromine concentrations (18.2 to 71.0 mg/L) were reached in animal samples between 2 and 24h followed by a decrease in urine, faeces, and rumen fluid, but remaining stable in blood and milk. At 48h, bromine concentrations ranged from 13.5 to 47.2 mg/L in animal samples. We hypothesise that repeated *Asparagopsis* dosing at ≤ 12 h intervals could increase bromoform, dibromomethane, and bromine levels in animal tissues and products before elimination. Results of the multi-dose experiment will provide further insights.

246 Potential of citrus essential oils for mitigation of enteric methane

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The burgeoning human population and the demand for animal protein have increased enteric methane emissions. Minimizing methane emissions, boosting sustainable production, avoiding chemicals as growth promoters, and providing consumers with quality products are critical for the global livestock industry. Essential oils (**EOs**) are complex mixtures of volatile (or aromatic) molecules, mainly terpenoids. Previous research has evaluated the use of EOs such as clove, garlic, oregano, thyme, and mint, among others, in feeding monogastric and some ruminant species. Evidence shows that EOs improve animal health and rumen fermentation, while reducing methane emissions. In the present study, the effect of essential oils of orange (**OEO**; *Citrus sinensis*), Persian lemon or lime (**PEO**; *Citrus x latifolia*), grapefruit (**GEO**; *Citrus x paradisi*), and limonene (**LMN**) obtained from orange (*Citrus sinensis*), on rumen fermentation and CH₄ production in vitro was evaluated. Doses of 50, 100, and 200 µl/g of each citrus essential oil and a control treatment without the inclusion of EO were evaluated. An integral ration with a 60:40 forage:concentrate ratio was used as substrate for the evaluations. Results showed an increase ($p < 0.05$) in the proportion of propionic acid in the three evaluated concentrations of OEO in the treatment of 200 µl/g of LMN, as well as in the concentration of 50 µl/g of LMN (28.68 %), in contrast with the control (25.78 %; $p < 0.05$). In the case of valeric acid, an increase was recorded for most of the doses evaluated ($p < 0.05$) except for the dose of 50 µl/g LMN. For acetic acid, reductions were recorded with 100 and 200 µl/g AEN?, 50 and 100 µl/g LMN, all evaluated doses of PEO, and 50 and 200 µl/g GEO ($p < 0.05$). Regarding gas production, an increase was recorded for all evaluated doses ($p < 0.05$) except for the 50 µl/g OEO dose. Methane (**CH₄**) production was reduced by 30%, 22.7%, and 10.7% at 50, 100, and 200 µl/g of OEO, respectively. On the other hand, the 50 µl/g dose of LMN reduced CH₄ production by 13 %. Only the 200 µl/g dose of PEO recorded a similar production to the control treatment, and the remaining doses of the EOs increased CH₄ production. Adding OEO (200 µl/g) reduced CH₄ production and increased the proportions of volatile fatty acids except acetate. Adding OEO to supplement beef cattle rations could be considered a natural alternative to reduce environmental impact. It is advisable to continue evaluating different doses across various substrates and to conduct in vivo studies using citrus essential oils.

28 Effects of Essential Oils and Forage Type on Methane Emissions and Dairy Cow Performance

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A completely randomised block design study investigated the effects of forage type and Anavrin (Vetos Europe SAGL, Switzerland), a blend of essential oils, bioflavonoids, and tannins, on methane emissions, dry matter intake (**DMI**), and performance in 64 lactating Holstein-Friesian cows at Wageningen Livestock Research (Dairy Campus, Leeuwarden, the Netherlands). Cows were blocked by parity, lactation stage, and milk yield, then randomly allocated to four treatments: corn silage (**CS**) or grass silage (**GS**), each with or without Anavrin supplementation.

Isonitrogenous and isoenergetic total mixed ration (**TMR**) diets were formulated to meet nutritional requirements with GS or CS as the main forage source and fed ad libitum. Anavrin (2 g/cow/day) was administered via experimental pellets, with placebo pellets for controls. The study comprised five phases: three-week covariate, one-week diet adaptation, two-week additive adaptation, and two three-week trial periods. DMI and body weight (**BW**) were recorded daily. Cows were milked twice daily, with milk yield measured at each milking and composition analysed weekly. Emissions were measured using GreenFeed systems (C-Lock Inc.) with palatable bait to encourage visits. Data were analysed using the MIXED procedure in SAS (v9.4, SAS Institute Inc., Cary, NC), with treatment, forage type, measurement period, and their two-way interactions as fixed effects, baseline measurements as covariates, and block and cow as random factors.

DMI and milk yield were unaffected by treatments. GS increased milk fat and protein content ($P < 0.0001$), while milk yield decreased from Period 1 to 2 ($P < 0.0001$). BW did not differ significantly between CS and GS ($P = 0.69$), increased in Period 2 ($P < 0.0001$), and tended to be slightly lower in cows receiving Anavrin ($P = 0.059$). Anavrin significantly reduced CH₄ (408 vs. 429 g/day; $P = 0.026$) and CO₂ production (14,766 vs. 15,243 g/day; $P = 0.003$). GS increased CH₄ production versus CS (434 vs. 403 g/day; $P = 0.001$). CH₄ production tended to be higher in Period 2 ($P = 0.083$), while CO₂ intensity increased ($P < 0.0001$). CH₄ yield was higher for GS than CS (23.9 vs. 21.9 g/kg DMI; $P < 0.0001$), with yields tending to be higher in Period 2 (23.2 vs. 22.5 g/kg DMI; $P = 0.062$). CH₄ intensity was higher for GS (17.2 vs. 15.8 g/kg FPCM; $P < 0.0001$) and increased from Period 1 to 2 (15.6 vs. 17.4 g/kg FPCM; $P < 0.0001$). Anavrin did not affect CH₄ yield or intensity.

No significant two-way interactions were observed between additive supplementation, forage type, and measurement period, indicating that Anavrin consistently reduced emissions regardless of the type of forage and duration of the supplementation. CS and Anavrin supplementation reduced CH₄ emissions without compromising milk production or composition.

267 Soybean expeller reduces enteric methane emissions from dairy heifers

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By-products from the cropping industry are commonly utilised as sources of protein and energy in ruminant diets. Substituting meal with expellers can modify diet energy source from starch to lipids. However, limited information exists on the impact of this feeding strategy on greenhouse gas emissions. The study aimed to evaluate the potential enteric methane mitigation, nitrogen excretion, ammonia emission and grain replacement of cattle supplementation. Two dietary treatments, with 60% whole-plant sorghum silage and 40% concentrate (on a DM basis), were offered *ad libitum* once daily to 12-one-year-old Holstein heifers (240±11 kg LW). Diets were formulated to be isoproteic (150 g CP/kg DM) and isoenergetic (2.5 Mcal ME/kg DM), differing on the lipids and starch content. The control diet (**CTRL**) contained maize grain:soybean meal (40:60), whereas the alternative (**EXP**) used maize grain:soybean expeller (20:80). A crossover design with two periods was used, with each heifer as experimental unit. After a 14-day acclimatisation, each period included 3 days of measurements. Heifers were weighed fortnightly, and feed amounts adjusted accordingly. Dry matter intake (**DMI**) was calculated as the difference between offered and refused feed during the last 3 acclimatization days and the measurement period. Enteric CH₄ emissions were measured using respiration chambers over 3 days per period. Following the first period, diets were switched. Faecal and urine samples were collected over 2 days at the end of each period for pooled analysis of nitrogen excretion and manure ammonia emission. Chemical composition was determined on the offered and refused feeds, faecal and urine samples. R Studio CrossCarry package and analysis of variance for crossover design were used for statistical analysis. No differences were observed on DMI (6.97 vs. 7.31 kg/d, 0.212 SEM, P=0.193), ME intake (17.6 vs. 17.8 Mcal/d, 0.462 SEM, P=0.454), and LW (285 vs. 282 kg, 3.86 SEM, P=0.82) of heifers fed EXP vs. CTRL diets, respectively. Heifers fed EXP had lower (P<0.0001) CH₄ production (159 vs. 191 g CH₄/d, 5.21 SEM), CH₄ intensity (0.559 vs. 0.675 g CH₄/kg LW, 0.014 SEM), and estimated Y_m (6.86 vs. 8.35 %, 0.217 SEM) than those fed the CTRL diet. These results could be explained by the higher intake of lipids (412 vs. 188 g EE/d, 0.007 SEM, P<0.0001) and lower intake of starch (702 vs. 1,049 g starch/d, 0.055 SEM, P<0.0001) of heifers fed EXP than CTRL diet. Replacing soybean meal with expeller in backgrounding diets reduced energy loss as enteric CH₄ by 18% and decreased maize grain dependence by 40%. So far, these results suggest that human non-edible food such as by-products from the cropping industry can reduce methane emissions without compromising food supply for human consumption. Analyses of nitrogen excretion and manure ammonia emission are still underway.

149 Effect of brewers' grains and rapeseed meal on methane emissions on four commercial dairy farms

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Previous research conducted at ILVO indicated that incorporating brewers' grains (**BG**) and rapeseed meal (**RSM**) into dairy cows' diet significantly decreased the carbon footprint while also effectively mitigating enteric methane emissions. These studies utilized precisely formulated diets, albeit with relatively high proportions of RSM and BG, which may exceed the practical application levels typically employed in commercial dairy farming operations. However, the efficacy of BG and RSM in alternative dietary compositions, particularly those with an elevated proportion of prewilted grass silage or corn silage, remains underexplored. Consequently, a series of trials was implemented across four commercial dairy farms with varying diets to evaluate the practicality and effectiveness of BG and RSM as a methane mitigation strategy in the Flemish dairy sector. A cross-over design encompassing five treatment periods, each lasting four weeks, was employed for the trials. The herds on each farm were split into two groups. These groups alternated between receiving one of three iso-proteic and iso-energetic dietary treatments: (1) a control diet reflecting the farm's usual feeding practice ©, (2) a modified control diet with increased inclusion of RSM (**T1**), and (3) a modified control diet incorporating both RSM and BG (**T2**). The sequence of treatments was administered as follows: C, C, T2, C, T1 for group 1, and C, T1, C, T2, C for group 2. Methane emissions were monitored using a GreenFeed system for each group. Daily milk production data were collected for individual cows, while milk quality was assessed once per period. Feed intake was recorded daily at the group level. Analysis revealed no significant differences in total dry matter intake among the treatments. The T2 treatment (BG and RSM combination) (441 ± 9.34 g CH₄/day) resulted in a significant reduction in methane emissions compared to both the control (452 ± 9.16 g CH₄/day, $p < 0.001$) and T1 (451 ± 9.33 g CH₄/day, $p = 0.001$) treatments, equating to a 2.4 % absolute reduction relative to the control. Methane yield was also significantly lower in the T2 treatment (20.1 ± 0.710 g CH₄/kg dry matter) compared to the control (21.3 ± 0.634 g CH₄/kg, $p = 0.023$), indicating a 5.6 % reduction. Furthermore, fat and protein corrected milk production was significantly higher in the T2 treatment (29.6 ± 0.760 kg) compared to both the control (28.3 ± 0.743 kg) and T1 (28.5 ± 0.760 kg) treatments ($p < 0.001$), with a 4.6 % increase over the control. Methane intensity was reduced by 8.1 % in the T2 treatment (15.8 ± 0.450 g CH₄/kg FPCM) compared to the control (17.2 ± 0.431 g CH₄/kg FPCM) and T1 (17.0 ± 0.450 g CH₄/kg FPCM) treatments across all farms ($p < 0.001$). In conclusion, the RSM treatment alone (T1) was ineffective as a methane reduction strategy. However, the combination of BG and RSM (T2) led to a modest absolute reduction in methane emissions. Importantly, the significant increase in fat- and protein-corrected milk yield resulted in an 8.1% decrease in methane intensity. These findings validate the combination of BG and RSM as a viable strategy for mitigating methane intensity in various Flemish dairy cow diets.

351 Emissions intensity from feedlot finished steers on a diet with maximum inclusion of ethanol coproducts

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The total emissions of greenhouse gases (**GHG**) of beef cattle finished in the feedlot as kg of carbon dioxide equivalent (**CO₂-eq**) can vary depending on several factors, including the ingredients used in their diets. The objective of this study was to evaluate the total intensity of emissions from two isoenergetic diets, one with regular ingredients used in Brazil (Conventional) and another formulated with the maximum inclusion of coproducts from ethanol production (Canapec), using a carbon footprint calculator. The data used were from an experiment conducted at Embrapa Pecuária Sudeste, São Carlos, SP, Brazil, with 40 Nellore steers distributed in 4 pens (n= 20/treatment). The Conventional diet consisted of 25.50% corn silage, 32.74% corn grain, 35.00% soybean hulls, 4.75% soybean meal, 1.00% urea, 1.00% mineral mixture, while the Canapec diet consisted of 13.50% sugarcane bagasse, 52.77% corn grain, 30.0% dry distillers' grains with solubles - DDGS, 0.66% peanut oil, 0.51% potassium chloride, 1.0% calcite, 0.56% urea, 1.0% mineral mixture (dry matter basis). To calculate the carbon footprint, in CO₂eq per kilogram of live weight, the Embrapa Low Carbon Livestock Calculator was used, based on the IPCC Tier 2 model, which follows the FAO/LEAP guidelines, meets the requirements of ISO 14.044 and the ICVCalc food library. Feedlot data were analysed using PROC MIXED from SAS, considering the treatments as fixed effects and the blocks (heavy/light animals) as random effects. The Conventional diet average daily gain was greater than Canapec [1.94 and 1.80 kg/head/day (P<.0001)], but Canapec intake was lower [11.10 and 9.54 kg DM/head/day (P<0.0001)]. For the calculation of emissions, detailed characteristics of the treatments were included, from animal performance to the nutritional composition of the diets and manure management, allowing the estimation of GHG emissions per live weight (**LW**), as (i) enteric, and (ii) feed production, and (iii) manure emissions, and total emissions. The total GHG intensity for the Control and Canapec treatments were 13.80 and 11.78 ± 0.006 kg CO₂-eq /kg LW, respectively. On average, 81% of total emissions were attributed to feed production, with 11.65 and 9.02 kg ± 0.001 CO₂-eq /kg LW; enteric emissions were 15%, corresponding to 1.836 and 2.068 ± 0.041 kg CO₂-eq /kg LW; and finally, 4% of the emissions are related to manure, 0.313 and 0.690 ± 0.057 kg CO₂-eq /kg LW, for the Conventional and Canapec treatments, respectively. Notably, the total emission intensity was lower for the Canapec diet, primarily due to the strategic selection of ingredients, effectively reducing emission intensity. Therefore, although enteric and manure emissions are slightly higher in the Canapec diet, its lower total intensity and higher feed efficiency highlight the importance of ingredient selection to reduce emissions in confinement systems.

309 Dihydrogen emissions were unaffected by alternative electron acceptors availability in rumen methanogenesis-inhibited dairy cows

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Inhibiting methanogenesis increase the concentration of dihydrogen in the rumen. This dihydrogen is generally not incorporated into useful metabolites for the host ruminant, so methanogenesis inhibition does not result in more feed-efficient and productive animals, hindering adoption of this type of mitigation strategy by farmers. We have shown in previous in vitro studies that when rumen methanogenesis was inhibited in vitro, simple phenolic compounds can act as dihydrogen acceptors and improve rumen fermentation. Gallic acid is a natural phenolic compound found in many plants that is converted to acetate by rumen microbes using H₂ or formate as an electron donor. In this study, we evaluated gallic acid as a dihydrogen acceptor in methanogenesis-inhibited lactating dairy cows. We hypothesized that combining the methanogenesis inhibitor *Asparagopsis armata* with gallic acid would stimulate phenolic-degrading rumen microbes to take up dihydrogen and produce more volatile fatty acids, resulting in improved milk production. This study used a randomized complete block design. Lactating Holstein cows (n=28) were blocked into 7 blocks with 4 animals each, based on parity, calving date, DMI, milk yield, and methane production during the covariate period. Within each block, cows were randomly assigned to one of the four treatment groups: **CON**, basal diet; **AS**, basal diet with 0.25% *A. armata*; **GA**, basal diet with 0.8% gallic acid; and **MIX**, basal diet with 0.25% *A. armata* and 0.8% gallic acid. The study included a 6-weeks covariate period and 5 weeks of experimental measurements. Dry matter intake, milk production and gas emissions were measured throughout the study. Rumen samples for fermentation and microbial analysis were collected at the end of the covariate and experimental measurement periods. The statistical model included *A. armata*, gallic acid, *A. armata* x gallic acid interaction, block, treatment x block interaction, and were adjusted by the covariate period. The addition of *A. armata* decreased methane yield by 25%, increased dihydrogen emissions threefold, and reduced the acetate to propionate concentration ratio in the rumen as compared to CON. In contrast, gallic acid did not affect methane or dihydrogen emissions, nor acetate or propionate concentration. Feed intake decreased by 10% in the AS group with a consequent decrease in milk yield of 18%. In the MIX group, feed intake was also decreased (8%), but the drop in milk yield was less severe (11%). This study, conducted with dairy cows, failed to corroborate our previous in vitro result on the effects of gallic acid on dihydrogen dynamics and volatile fatty acid when methanogenesis was inhibited. Our previous in vitro results indicated a remodelling of the microbial community when phenolic compounds were supplied in a continuous manner. The ongoing metataxonomic and metagenomic sequencing analysis aims to identify microbial features that are responsible for the observed discrepancies. Those results are underway and will be presented at the GGAA conference.

55 Linking dietary garlic intervention to methane emissions reduction in small ruminants: Evidence from meta-analysis

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Methane emissions from ruminants are of major concern, as they account for 16% of global greenhouse gas emissions and 2-12% of dietary energy loss. In this sense, reducing its production would lead to more efficient use of feed energy and reduce methane emissions to the environment. Thus, modulating rumen fermentation in ruminants through nutritional approaches offers an opportunity to mitigate greenhouse gas emissions and global warming. The use of dietary garlic, one such nutritional strategy to modulate rumen fermentation and reduce methane gas production in small ruminants, has yielded inconsistent results, thus making it difficult to use this information in decision-making. As a result, this meta-analysis was designed to assess the effectiveness of garlic intervention to improve rumen fermentability and reduce greenhouse gas emissions in small ruminants. PubMed, Web of Science, Google Scholar, and Scopus databases were searched for articles on the topic using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (**PRISMA**) guidelines. Eligibility criteria for an article to be included in the meta-analysis were information on moderators [small ruminant type, body weight, sex, breeds, diet types, duration of garlic intervention, and quantity of garlic added to the diet] considered a priori to influence rumen fermentation in small ruminants. Nineteen studies met the eligibility criteria and were used for the meta-analysis. Analyses were done in Open Meta-analyst for Ecology and Evolution (**OpenMEE**) software and the R package. Response variables [ruminal pH, total volatile fatty acids (**TVFA**), acetate, propionate, butyrate, acetate/propionate ratio, ammonia nitrogen, and methane emissions] were pooled using the random-effects models, and results were presented as mean differences (**MD**) at 95% confidence intervals (**CIs**). Heterogeneity was assessed using standard methods, whereas presence of publication bias was examined using the Egger and Begg tests. Subgroup analyses were conducted to explore the small ruminant type on methane emissions. Meta-regression analysis was performed to quantify the individual impact of moderators on the observed heterogeneity on methane emissions. Results suggest that dietary garlic intervention at 0.25 to 7.00% increased ($p < 0.05$) TVFA, acetate, and propionate in small ruminants. In contrast, pooled analysis showed that the addition of 0.25 to 7.00% garlic to the diets of small ruminants reduced ruminal methane emissions by 6.96% ($p < 0.05$) without evidence of publication bias, indicating that pooled results were valid and reliable. Subgroup analyses results revealed that dietary inclusion of garlic at 0.25 to 7.00% reduced ruminal methane emissions in sheep and goats by 6.21% ($p < 0.05$) and 8.23% ($p < 0.05$), respectively. Meta-regression results revealed that small ruminant type, breeds, diet types, and body weight explained most of the inconsistent results across studies that evaluated the effect of garlic on methane emissions in small ruminants. It is concluded that dietary garlic intervention at 0.25 to 7.00% can effectively improve rumen fermentation efficiency and reduce methane emissions in small ruminants. In addition, the results will help ruminant nutritionist, farmers, and policymakers in making informed decisions on the use of garlic to improve rumen fermentation efficiency and reduce methane emissions in sheep and goats.

105 Freeze-dried *Lactobacillus spp* improved daily weight gain and reduced methane intensity in growing lambs

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Direct fed microbials are natural enteric-methane mitigant options with no discernible residues in animal products. While previous studies have demonstrated the effect of *Lactobacillus spp* on methane mitigation both *in vitro* and *in vivo*, it remains uncertain how to effectively deliver direct fed microbials in a free-grazing environment while also improving productivity and minimising methane emissions. The objective of this experiment was to evaluate the effect of precisely feeding a freeze-dried, direct fed microbial product comprising *Lactobacillus spp* to growing lambs on methane emissions and intensity, and liveweight gain. One hundred post weaning lambs were selected from a mob of terminal sired lambs born from maternal composite ewes in July 2023. Lambs were adapted to a standard grain mix over 14 days and then introduced to an automatic feeder (C-Lock Super SmartFeed™, C-Lock Inc., Rapid City, South Dakota, United States) over the following 21 days. The automatic feeder used radio frequency identification ear tags to identify individual lambs and dispense their daily allocation of feed, enabling a precise amount of feed to be provided in multiple portions throughout the day. Following adaptation to the feed and the automatic feeder, 68 lambs (42.1 ± 3.2 kg body weight) that were well adapted to the automatic feeder were used for the remainder of the experiment. Lambs were randomly allocated to one of the two groups: a control group receiving a standard grain mix, and a treatment group receiving the same grain mix with a freeze-dried *Lactobacillus spp* supplement (MYLO®, Terragen Biotech Pty Ltd, Coolumb Beach, Queensland, Australia), delivering 1 x 10¹¹ CFU/kg of feed, with treatments fed for 70 days. The initial grain allocation was set at 450 g/head per day and was gradually increased over a 14-day period, reaching 1.1 kg/head per day. Animals had *ad libitum* access to water and vetch (*Vicia sativa* L.) hay throughout the experiment. Individual lambs were weighed weekly. Methane was measured using portable accumulation chambers on days 31, 59 and 70 of the experiment. The average methane production and methane intensity over the experimental period was calculated as the simple mean of the three measurement periods. Data were analysed using randomised complete block design-based analysis of covariance. Lambs in the *Lactobacillus spp* supplemented group had a higher average daily weight gain (P=0.009) by the end of the study compared to the control group. The average methane production across all three measurement periods did not differ between the treatment groups (21.7 g/d, P = 0.095). Methane intensity for lambs fed the *Lactobacillus spp* supplement was lower than the control group (0.14 v/s 0.20 g/g average daily weight gain, P=0.008). *Lactobacillus spp* supplementation fed to growing lambs on a hay-based diet improved liveweight gain and lowered methane intensity over a 70-day feeding period. Moreover, the application of an automatic feeder technology enabled precise feed allocation to individual animals within a larger group of animals, showcasing its potential for efficient delivery of dietary methane mitigants.

193 Effect of dietary fat supplementation on methane emissions from dairy cows in Fiji

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Dietary interventions to reduce methane emissions from ruminants can improve animal productivity. The objective of our research was to determine the effects of fat supplementation on total methane production (g d^{-1}), methane yield (g kg-DMI^{-1}), and methane intensity (g kg-ECM^{-1}) from cows on a commercial Fijian dairy farm. We hypothesized that cows fed a low-fat diet would have greater methane yield and methane intensity than cows fed a high fat diet. The research was conducted at a commercial dairy farm in Tailevu province, Fiji. Twenty cows of mixed breed, 363 ± 50.3 body weight, and 4.1 ± 1.14 years of age were enrolled in the experiment. The experiment was designed as a crossover with 2 periods and 2 diets. Each period was 19 days with a transition to diet (days 1 to 3), adaptation to diet (days 4 to 13), and measurement period (days 14 to 19). The diets were 1) a low-fat diet (target 20g kg^{-1} fat; forage plus mill mix, 1 kg-DM d^{-1} ; wheat flour, 0.5 kg-DM d^{-1} ; molasses, 0.5 kg-DM d^{-1} ; pea meal, 4 kg-DM d^{-1} ; soymeal, 1.5 kg-DM d^{-1}) and 2) a high-fat diet (target 50 g kg^{-1} fat: forage plus mill mix, 1 kg-DM d^{-1} ; copra, 5 kg-DM d^{-1} ; molasses, 0.5 kg-DM d^{-1} ; soymeal, 1 kg-DM d^{-1}). Both diets used the same basal forage of paragrass, siteria and juncao (3 kg-DM d^{-1}). During the measurement, the concentrate portion of each diet was offered in dairy, and forage was offered in individual stalls to enable measurement of intake. Methane production was measured using the sulphur hexafluoride tracer technique with gas analysis by chromatography. Data was analysed by ANOVA with a complete blocking structure of (cow by cycle) within cohort, using Genstat 24.

Fat concentration was 22 g kg^{-1} in the low-fat diet and 66 g kg^{-1} in the high-fat diet. Cows offered the low-fat diet had greater feed intake than the cows offered the high-fat diet (11.8 vs 9.8 kg d^{-1} , $P < 0.001$). Energy corrected milk yield was not affected by treatment (12.0 kg d^{-1} , $P = 0.750$). Cows offered the high-fat diet had lower methane production (245 vs 201 g d^{-1} , $P = 0.003$) and lower methane intensity (21.8 vs $18.0\text{ g kg-ECM}^{-1}$, $P = 0.049$). Methane yield was not affected by treatment ($21.0\text{ g kg-DMI}^{-1}$, $P = 0.921$).

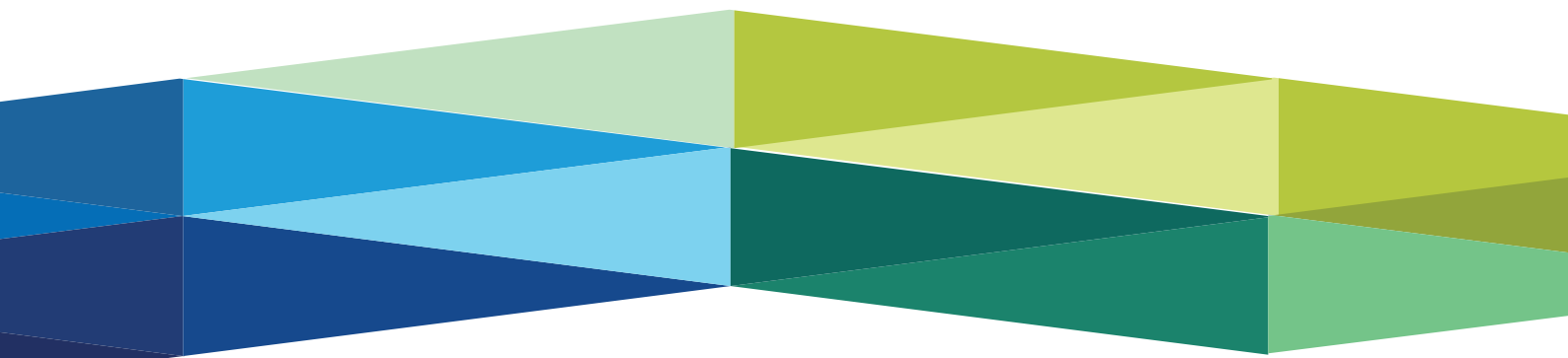
Feeding a high-fat diet to Fijian dairy cows reduced methane production and methane intensity by 18 %, respectively, however, feed intake was also reduced by 18%.



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1.2 Dietary supplementation to reduce ruminant greenhouse gas emissions – Feed additives (part II)



384 Effect of SeaFeed™ oil on finishing beef cattle methane output, performance and meat quality

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The red seaweed *Asparagopsis armata* contains the bioactive compound bromoform (**CHBr₃**) which has been shown to be highly effective at inhibiting the production of enteric methane (**CH₄**) in ruminants. CHBr₃ is volatile and suspension in rapeseed oil has been shown to increase stability and shelf-life. SeaFeed™ (**SF**) contains *A. armata* extract in rapeseed oil as a CHBr₃ delivery mechanism. The aim of the current study was to evaluate the effectiveness of SF at two inclusion rates when included in a grass silage-based ration to finishing beef cattle. The secondary aim was to evaluate the bromoform content in animal tissues to assess effects on end-product quality. Fifty-four beef origin finishing cattle were blocked by breed, sex and body weight (**BW**) and randomly allocated to a treatment group: 0% (Control, n=18), 0.25% (Low SF, n=18), and 0.5% (High SF, n=18) of SF based on dry matter intake (**DMI**) for the 108d study duration. CH₄ measurements were collected using the GreenFeed system in 14d intervals, BW was recorded on 0d and every 14d, individual DMI was recorded daily to provide weekly averages. At the point of slaughter, rumen fluid was collected for further analysis (NH₃-N, VFA, pH, Metagenomics). Carcass weight, back fat depth (3 points/animal) and carcass colour scores were recorded. CHBr₃ quantification was carried out in triplicate using GCMS-MS following double methanol extraction from freeze-dried samples of loin, liver and kidney fat. CH₄ measures were predicted as a mixed linear model using the REML commands in Genstat. Fixed effects included age, breed, sex, feed conversion rate and treatment. Pen was included in the random model. Rumen fermentation and CHBr₃ analysis was completed using version 4.3.2. of 'R' and version 4.3.1. of 'RStudio'. Significant differences between means were determined by repeated-measures ANOVA, followed by post-hoc paired t-tests with Bonferroni correction (p<0.05). No significant differences were observed for DMI, ADWG, body condition score (P>0.05) and animal health (P>0.05) across treatment groups. CH₄ expressed as CH₄ g/d, CH₄ g/kg DMI and CH₄ g/kg LWG was significantly different across treatment groups with a dose response being observed (P<0.001). Values of 29.02, 25.42 and 21.12 (SD = 1.16) were recorded for CON, Low SF and High SF respectively for CH₄ g/kg LWG. Rumen acetic acid (Mmol/L) significantly reduced with increased SF dose being 60.24, 58.54 and 57.21 respectively (SD = 2.30, P<0.05) and rumen propionic acid (Mmol/L) significantly increased with elevated SF dose being 15.93, 16.84 and 17.31 respectively (SD = 1.48, P<0.05). CHBr₃ was negligible across all tissue samples tested (< 0.06mg/g) with no significant differences observed across treatments (P>0.05). The study was the first to investigate the potential of SF in a grass silage-based diet as a CH₄ mitigator for beef cattle. The results show that SF could be incorporated without negatively affecting animal performance or end-product safety whilst significantly reducing CH₄ output. Future works include completion of metagenomic analysis on rumen fluid samples and evaluation of forage mixing method on CHBr₃ stability for differing feeding systems.

466 Phytosterol supplementation reduced ruminal methane emissions and improved lactation performance in dairy cows

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As a potent greenhouse gas, methane has a warming potential up to 28 times greater than that of carbon dioxide, contributing approximately 14% to total global greenhouse gas emissions. Ruminants are the primary source of CH₄ emissions in animal husbandry, with CH₄ primarily generated through rumen microbial fermentation, leading to a loss of dietary energy in ruminants. Phytosterols, a class of active steroid compounds naturally present in plant cell membranes, have recently been found to regulate energy metabolism and gut microbiota. This study aims to investigate the effects of phytosterol supplementation on lactation performance and methane emissions in dairy cows, revealing its underlying mechanisms to provide a theoretical basis for developing environmentally friendly dairy cow diets. Thirty-four multiparous Holstein dairy cows were selected for this study based on their similar days in milk (207 ± 23) and milk yield (35.4 ± 2.7 kg/d). The cows were randomly assigned to 2 groups, receiving either 0 g or 15 g commercial phytosterols per day. All cows had free access to drinking water and a total mixed rations formulated according to NRC (2012). The phytosterols used in this study, commercial named NASTEROL, was obtained from Nanjing Nature Bio-Tech Co., Ltd. (Jiangsu, China). The experiment lasted for 50 days with 7 days for adaptation and 43 day for trial. Sample collection occurred from day 30 to day 43 of the trial phase, which included 5 days for milk sample collection and 8 days for CH₄ emission determination. Milk sample were added with potassium dichromate tablets immediately after collection and mixed proportioned according to morning, afternoon and evening milk production (4:3:3), followed by milk composition analysis (FOSS-4000, Denmark). Milk production from each cow was recorded daily throughout the experimental period. Furthermore, 7 cows from each group were randomly selected to measure ruminal gas yield using an automated head-chamber device. Automated head-chamber device is a mobile “bait” station similar to GreenFeed that collects gas with negative pressure and continuously monitors gas concentrations with sensors. For each cow, measurements were taken at eight time points throughout the day: 01:00, 04:00, 07:00, 10:00, 13:00, 16:00, 19:00 and 22:00, repeat it once, followed by calibration and calculation. Data were analyzed using independent-sample t test of SP SS 20.0. Significance level was declared at $P < 0.05$. Dry matter intake, milk yield, and energy-corrected milk increased by 17.91%, 14.85%, and 24.04%, respectively. Regarding milk composition, the concentrations of fat, protein, total milk solids, and non-fat solids rose by 17.11%, 5.22%, 7.10%, and 3.16% ($P < 0.05$). Milk sugar differences between groups were not observed. Regarding greenhouse gas emission, the production of rumen CH₄ and CO₂ per kg of dry matter intake with phytosterols decreased by 16.93% and 16.03%, respectively ($P < 0.05$). Additionally, CH₄ production per kg of energy-corrected milk was significantly reduced by 14.42% ($P < 0.01$). As a result, feeding phytosterols shows that cows can more efficiently utilize energy for lactation, improve milk quality and reduce methane emissions. This study potentially offers new methane reduction strategies for dairy cows.

136 Evaluating Rumin8 as a feed additive to mitigate methane emissions in beef cattle

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The livestock sector is a significant contributor to global greenhouse gas emissions, with enteric methane (**CH₄**) production from ruminants being a primary source. Feed additives containing bromoform (**CHBr₃**) have shown promise in reducing CH₄ emissions while minimizing impacts on animal productivity. This study investigated the efficacy of two synthetic CHBr₃-based Rumin8 Investigational Veterinary Products (**IVPs**) in reducing enteric CH₄ emissions, alongside their effects on animal performance and rumen health, in feedlot cattle. The study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis (Protocol No. 23094). Twenty-four Angus steers were randomly assigned to one of three treatment groups: Control, Oil (8 mL Rumin8 oil IVP/kg dry matter intake, **DMI**), and Powder (1.2 g Rumin8 powder IVP/kg DMI). The Oil IVP consistently delivered 32.2 mg CHBr₃/kg DMI, while the Powder IVP provided 2.0 mg CHBr₃/kg DMI during weeks 1–8, increasing to 17.9 mg CHBr₃/kg DMI from week 9 due to a reformulated batch. Over the 12-week trial, methane emissions were assessed using the GreenFeed system. All analyses were conducted in R statistical software using Linear Mixed-Effects models (nlme and lme4 packages). Six model configurations were tested to address variation, including random effects, covariates, heteroscedasticity (varPower, varIdent), and autocorrelation (corCompSymm, corAR1). The best-fitting model for each variable was selected based on residual normality and the lowest Akaike Information Criteria. The Oil group showed reductions in CH₄ production (–95.2%), yield (–93.0%), and intensity (–93.4%) compared to the control group. These reductions were accompanied by significant increases in hydrogen emissions. By contrast, the Powder group showed no significant impact on CH₄ parameters despite an increased CHBr₃ concentration in the latter part of the trial. Neither treatment adversely affected key animal performance metrics such as average daily gain or feed conversion efficiency. Rumen health parameters, including pH and volatile fatty acid profiles, also remained unaffected. This study provides evidence supporting the potential of synthetic bromoform-based feed additives as scalable solutions for reducing the environmental impact of livestock production. Further research is warranted to optimize dosing strategies and evaluate long-term sustainability impacts.

461 Use of Bovaer as antimethanogenic additive in drinking water for grazing cattle

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Technologies that significantly reduce enteric methane emissions from ruminants need to be developed for most grazing operations to reach a carbon neutral position. Recently, 3-nitrooxypropanol (**3-NOP**, commercial name Bovaer®) has been developed as feed additive for ruminants for inhibiting methane production in the rumen. Novel ways of delivering the compound to grazing cattle are currently under development. Encouraging results with 3-NOP applied in drinking water have been obtained recently using a small ruminant animal model in respiration chambers. This study aims to evaluate the effectiveness of water-based delivery of 3-NOP in decreasing methane emissions in grazing cattle.

Sixty *Bos indicus* steers (mean body weight $\pm$ SD: 287 $\pm$ 15.1 kg) grazing tropical pastures at Lansdown Research Station (Queensland, Australia) were allocated to two treatment groups: control (no 3-NOP supplementation) or 3-NOP (target 30 mg 3-NOP per Liter of water, equivalent to 2.5 mg 3-NOP kg⁻¹ body weight). The study was conducted over a 60-day period in four independent replicated paddocks. Four Smartdosing+ D25+ units were used to deliver the treatments through drinking water and quantify herd-level water intake. Methane and hydrogen emissions were measured using four portable GreenFeed systems (C-Lock Inc., Rapid City, SD, USA). Additionally, each animal was fitted with an eGrazor smart collar integrating tri-axial accelerometer and a Global Navigation Satellite System (**GNSS**) receiver to monitor and analyse individual behaviour patterns and locations. The inclusion of the 3-NOP in drinking water had no significant effect on body weight compared to control group ($P > 0.05$). Methane and hydrogen production from animals receiving the treatment through drinking water were significantly different to control animals ($P \leq 0.05$). Methane production was reduced by up to 27% while hydrogen emissions increased by up to 5.4-fold in treated animals. Accelerometer data collected from the eGrazor collars was used to classify individual cattle behaviour at each five-second interval as grazing, ruminating, resting, walking, drinking or other. The predicted behavioural data was then utilised to estimate daily dry matter intake. Further, the behaviour profiles were used to determine when the consumption of 3-NOP occurred relative to other behaviours. The typical behaviour profile for cattle included two intensive periods of grazing from 5-8 AM and then 3-7 PM. The period outside of the grazing peaks is largely characterised by ruminating and resting behaviours. Peak drinking behaviour was observed at 6 AM, 10 AM and 3 PM and occurred shortly after peak grazing periods. The findings suggest that Bovaer® can be effectively delivered through drinking water under appropriate conditions, offering a viable methane mitigation strategy for grazing livestock. The observed variability in methane reduction was associated with natural fluctuations in water intake leading to variations in 3-NOP ingestion by the animals. Importantly, no adverse effects on animal performance were observed. Further analyses are focusing on integration of grazing behaviour and timing of consumption of the antimethanogenic compound influence its effectiveness.

427 Improved productivity and enteric methane mitigation following molasses block supplementation of Cambodian smallholder beef cattle

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Livestock raising is important in Cambodia, with an estimated 3.4 million cattle located in 780,000 smallholder farming households, although productivity is low, constrained by poor animal nutrition, health and management. This study examined supplementation of cattle with 20 kg 'Emissions Control Blocks' (**ECB**, AgCoTech Laos) for improved growth rates and reduced enteric methane production. Smallholder farmers (n=40) within two villages in Pursat Province each provided two age and weight matched local breed cattle (n=80). One animal from each of the 40 farms was designated 'treatment' and accessed the ECB for a 10-week duration, with 'control' animals denied access. To assess production impacts and calculate Emissions Intensity (**Ei**), regular measurements included body weight by tape to calculate average daily gain (**ADG**), body condition score (**BCS**), and ECB consumption rates. At the end of the trial, breath methane concentration was measured by a hand-held CH₄ monitor in both groups, enabling a recording of direct methane abatement. Results of Ei and the direct abatement measured with the methane laser were then summed ('stacked') to calculate the total CH₄ abatement and estimated annual GHGe impact of ECB per animal per year. The average body weight of the 'treatment' cattle increased by 4.4 kg (ADG of +63 g/day) compared to a decline by 1.1 kg (ADG of -16 g/day) in 'control' animals (P < 0.05). A 'dose-response' effect of ECB consumption was observed, with increased body weight by 0.33 kg/kg ECB consumed in the first 5 weeks (P = 0.036) compared to 1.33 kg/kg ECB consumed in the second five-week period (P = 0.002). BCS differences between groups were not observed. Modelling the Ei using IPCC inventory software indicated the block reduced CO₂e emission from a Business as usual or baseline of 1320 to 1100 kg per annum. The effect of the ECB (P = 0.0019) on enteric or breath methane concentration was confirmed, with 'treatment' estimated to produce 88.0 g/day compared with 106.0 g/day for 'control', a 17.0% decrease in daily CH₄ production and extrapolated to 184 kg CO₂e per annum. On stacking direct abatement and the IPCC calculation of reduced Ei, total estimated annual GHGe reduction achieved was 30.6%. These findings occurred despite lower ECB consumption rates than expected, attributed to excessive hardness of this batch of blocks. It was concluded that ECBs offer both productivity gains with Ei and direct enteric methane abatement in cattle.

The authors confirm that the research to be reported will be completed, and shared at the time of the conference.

61 Environmental impact of using Actisaf® Sc 47 as feed additive in dairy and beef cattle.

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Today, one of the major challenges of dairy and beef producers is to reduce their environmental footprint to establish more effective, efficient, and sustainable production systems. One of the solutions to achieve these objectives is to use feed additives such as yeast probiotic. The aim of the study was to estimate the environmental impacts of producing 1 kg of fat- and protein-corrected milk in dairy cows and 1 kg of body weight gain in fattening beef cattle with and without Actisaf®Sc 47 supplementation. For dairy cows, three trials were selected: French, UK and German trial. During each trial, two groups of cows were compared: control without supplementation and experimental with Actisaf®Sc 47 supplementation. Supplementation was carried out from calving to 108 and 120 days of lactation in the French (5g/cow/d in top dressing) and the UK trial (10 g/cow/d mixed with the basal ration), respectively. For the German trial, supplementation (5g/cow/d mixed with the basal ration) started from 109 days of lactation up to 201 days. For beef cattle, four trials were selected: Italian trial A, Italian trial B, Spanish trial and French trial. During each trial, two groups of animals were compared: control without supplementation and experimental with Actisaf®Sc 47 supplementation at 5g/day. For both species and for each trial, ration composition, dry matter intake (**DMI**), feed efficiency, milk quantity and quality in dairy and growth performance in beef were measured. The impact assessment was performed using SimaPro 9.3 by Blonk consulting for dairy and EVEA consulting in beef. In dairy cows, during the period of supplementation, Actisaf Sc 47 reduced carbon footprint up to 5%. Reported on a yearly scale including all lactation cycle, dry cows, and periods with and without supplementation, the use of Actisaf®Sc 47 during the three trials reduced, in average, the carbon footprint, land use, water use, resource use, acidification, freshwater eutrophication, marine eutrophication, and terrestrial eutrophication by 2.9, 2.05, 2.47, 1.67, 2.28, 2.18, 2.14, and 2.28%, respectively. In beef, supplementation with Actisaf®Sc 47 reduced the climate change impact from 3.8 to 6.6%, particulate matter from 4.1 to 7.1%, acidification from 4.1 to 7.1%, freshwater eutrophication from 3.9 to 8.2%, marine eutrophication from 4 to 6.4%, terrestrial eutrophication from 4.1 to 7.1%, land use from 3.9 to 6.2%, water use from 3.4 to 5.9%, and fossil resource use from 3.7 to 7.3%. The supplementation of Actisaf®Sc 47 showed very low contribution to the total carbon footprint, ranging from 0.005 to 0.016%.

The beneficial effects of Actisaf®Sc 47 on environment may be attributed to the positive effect on performance and feed efficiency. Hence, due to multiple positive effects, the use of Actisaf®Sc 47 is highly recommended in dairy cows and beef cattle.

407 Inclusion of peanut skin to the diet on enteric methane emissions in sheep fed alfalfa

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Peanut is an important crop for Córdoba province (Argentina) which generates *circa* 10,000 tonnes annually of skin (tegument), which can be used as animal feed. Peanut skin contains polyphenols, diverse and complex compounds known to inhibit methanogenesis. This study aimed to determine the effects of peanut skin inclusion on enteric methane emission in sheep fed alfalfa. Twenty Poll Dorset rams (47.6 ± 5.7 kg BW) were blocked by weight and randomly assigned to three levels of peanut skin inclusion (DM basis): 0% (CONTROL; $n = 6$), 10% ($n = 7$) and 20% ($n = 7$). Rams were kept in individual pens, and fed alfalfa and peanut skin, both pelletized, once a day in a single feeder. Peanut skin (29, 318 and 160 g kg⁻¹ of N, NDF and fat; 65 mg GAE g⁻¹DM) was gradually incorporated (10 days) replacing the alfalfa (34, 466 and 37 g kg⁻¹ of N, NDF and fat). Methane emissions were assessed 35 and 85 days after achieving the targeted inclusion levels. During methane measurement, feed offer was restricted to approximately 0.9 of the *ad libitum* dry matter intake (42.5 g kg⁻¹BW). Methane emission was determined using SF₆ tracer gas technique, adapted for an extended duration (4 days) of exhaled gas samples collection. Calibrated SF₆ permeation tubes (release rate: 2.027 ± 0.823 mg d⁻¹) were inserted 10 days before measuring methane emission (5, 6 and 5 animals for 0, 10 and 20% treatments). Three canisters sampled air to correct emissions for background gases. Gases concentrations were determined by chromatography. Animals were weighed unshrunk at the beginning, every 14-21 days, and at the end of the experiment (95 days) to calculate average daily BW gain and to adjust feed supply. Data was analysed by ANOVA with a mixed model for a complete randomized block design considering animals as a random effect. Treatment means were compared by pre-planned contrasts (CONTROL vs. TREATMENT; 10% vs. 20% inclusion levels). Data analysed for intake and methane is preliminary (only the first period). Peanut skin inclusion had no effects on DM intake (2.8 ± 0.30 kg d⁻¹; 42.0 ± 1.30 g kg⁻¹BW), but increased daily gain ($P = 0.0219$), being 197, 218 and 253 (± 13) g d⁻¹ for 0, 10 and 20% respectively; and gain tended to be higher with 20% compared to 10% inclusion ($P = 0.0620$). Methane emissions were not affected by peanut skin inclusion ($P > 0.1$), either as absolute values ($53.8, 42.7$ and 43.9 ± 7.70 g CH₄ d⁻¹) or relative to intake ($17.6, 15.2$ and 15.4 ± 2.64 g CH₄ kg⁻¹DMI for 0, 10 and 20% inclusion respectively). This was probably due to a high variability and small replicate numbers. Methane intensity was reduced ($P = 0.0244$) in rams receiving peanut skin ($275, 193$ and 163 ± 31.6 g CH₄ kg⁻¹ gain for 0, 10 and 20% inclusion respectively). In conclusion, peanut skin inclusion to an alfalfa diet does not affect intake, improves gain and reduces 30% methane intensity in sheep.

120 Early-life *Asparagopsis taxiformis* oil supplementation reduces methane emissions in dairy calves

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Modifications in rumen microbial colonization before weaning can have enduring effects on the rumen microbiome, influencing phenotypes later in life. This study evaluated the impact of early-life *Asparagopsis taxiformis* in oil supplementation on methane emissions (g/day) in Holstein bull calves. Sixteen calves were assigned at birth to one of three treatment groups: Control, Short-term, or Long-term. In the Short-Term group, supplementation began on the second day of life and continued until week 12, which is three weeks post-weaning. The supplement was provided with the calf concentrate, with dosage gradually increasing over time. For the Long-Term treatment, supplementation started at week 10, aiming to provide 16 mg of bromoform/kg of dry matter intake, with no ramp-up period. Methane emissions were measured during the post-weaning period (weeks 10–20) using the GreenFeed system, with data averaged biweekly per animal. Data were processed in two stages. First, outliers were identified using the interquartile range method, removing five weekly records before model fitting. Second, inverse data transformations and additional outlier removal (two of the biweekly data) were performed to ensure the normality of residuals. Linear mixed-effects models were used to evaluate treatment, age effects, and their interactions, with an autoregressive correlation structure accounting for repeated measurements. Global ANOVA results indicated a significant overall effect of treatment ($P = 0.0364$). Short-Term calves showed a sustained reduction in methane emissions after the cessation of bromoform administration. Both treated groups produced less methane than the control group (–22.5% and –17.4% for Short- and Long-term treatments, respectively). However, only the reduction observed in the Short-term group was statistically significant ($P = 0.046$). These results underscore the potential of early dietary interventions to achieve sustained reductions in methane emissions, offering a promising strategy for improving environmental sustainability in livestock production.

327 *In vitro* screening of native and non-native European trees, shrubs, and herbs for methane reduction

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Enteric CH₄ emissions from ruminant livestock significantly contribute to climate change, necessitating mitigation strategies. Alternative forages, including trees, shrubs, and herbs, have shown promise for reducing CH₄ emissions due to their secondary plant metabolites. This *in vitro* study evaluated 45 plant species harvested in Flanders, Belgium, in July 2022 for their CH₄-reducing potential, focusing on absolute CH₄ production (μmol/g DM), total SCFA production (μmol/g DM), and relative CH₄ production (mol CH₄/mol total SCFA). Leaf traits were assessed using the TRY database. Twelve promising species were selected for a second experiment involving fresh and ensiled substrates, tannin-binding polyethylene glycol assays, and varied *in vitro* headspace gas conditions (100% CO₂ vs. 50% CO₂:50% H₂). The first experiment revealed that while some species nearly eliminated CH₄ production, this was often accompanied by a significant reduction in SCFA production. *Hedera helix* uniquely achieved a 59% reduction (P<0.001) in absolute CH₄ production without affecting total SCFA production. *Castanea sativa* exhibited the lowest CH₄ and SCFA production, correlating with its high phenolic content. Absolute CH₄ and total SCFA production were negatively correlated with leaf C (r=-0.39 and r=-0.45) and leaf DM (r=-0.44 and r=-0.52). However, these Pearson correlations disappeared in relative CH₄ production, indicating a closer association of leaf traits with overall fermentability than specific CH₄-mitigating potential. In the second experiment, species such as *Castanea sativa*, *Catalpa bignonioides*, *Alnus glutinosa*, *Populus nigra*, and *Hedera helix* demonstrated the strongest anti-methanogenic effects, likely attributed to compounds like tannins, saponins, and quercetin. Ensiling reduced total phenol concentrations by up to 50% in most species, diminishing CH₄ mitigation potential, except for *Alnus glutinosa*, which consistently reduced CH₄ across fresh and ensiled substrates. Polyethylene glycol assays confirmed the role of tannins in CH₄ reduction by binding tannins and mitigating their effects on fermentation. However, polyethylene glycol was ineffective in incubations with *Castanea sativa* and *Catalpa bignonioides*, despite the high phenol concentrations observed in *Castanea sativa* and previous reports demonstrating the CH₄-mitigating effects of chestnut tannin extracts. This inefficacy is consistent with the fact that polyethylene glycol primarily interacts with condensed tannins, whereas *Castanea sativa* predominantly contains hydrolysable tannins. Headspace H₂ experiments further confirmed direct methanogen inhibition by certain species, particularly *Castanea sativa*, *Catalpa bignonioides*, *Hedera helix*, and *Alnus glutinosa*. Adjustments to the *in vitro* setup, including elevated headspace H₂ and polyethylene glycol supplementation, provided insights into the mechanisms underlying CH₄ reduction. However, detailed phytochemical and microbiological analyses are needed to fully elucidate these effects. Future studies should also explore the practical potential of these forages in livestock diets through *in vivo* trials and mixed-diet evaluations.

231 Variation in individual cow's response to methane-reducing additives

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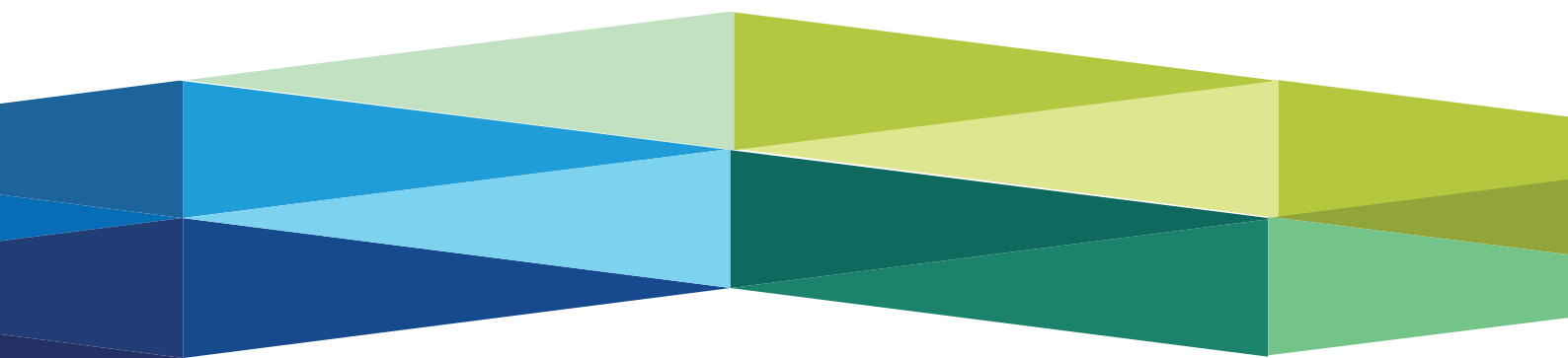
Dairy cows vary considerably in methane yield (CH_4/kg dry matter (**DM**) intake (**DMI**)), but it is unknown whether they also vary in response to methane-reducing additives. An earlier study indicates that high-methane emitting cows respond less to methane-reducing additives than low-methane emitting cows. This study aimed to analyze how individual cows varied in methane yield response to methane-reducing additives and whether responses were related to cows' methane yield and other phenotypic traits. Experimental treatments were, besides control, two methane-reducing additives: 3-nitrooxypropanol (**3-NOP**) and nitrate. A total of 186 first-lactation Danish Holstein cows were used in a 3×3 Latin square design, where cows entered the study individually 2-3 weeks after calving, with each period lasting 21 days. A basal diet was based on (% of DM basis) 55 forage (mix of 50:50 maize:grass clover silage), 18 rape seed meal, 15 barley, 10 sugar beet, and 2 minerals and vitamins. Treatments were added to the basal diet at 60 mg 3-NOP/kg DM (Bovaer) or 8.5 g nitrate/kg DM (Silvair). The enteric gas emissions (CH_4 , CO_2 , H_2) were measured using two GreenFeed units (C-Lock Inc.), and cows were offered max 1.1 kg DM concentrate daily in the GreenFeed as bait. Ruminal content was collected using an oral-ruminal probe (Ruminator) to determine volatile fatty acid (**VFA**) concentrations. Feed intake was recorded with the Insentec feed bins (Hokofarm Group). Cows were milked twice daily and sampled one day per week for milk composition analysis. Data for statistical analysis were obtained during the last week of each experimental periods for individual cows. The random cow effects (**RCE**) were extracted using a mixed model in SAS, which included Treatment (T, $n = 3$), Period (P, $n = 3$), P $\times$ T, and Experimental week number ($n = 81$) as categorical fixed effects, and Cow ($n = 186$) as random. Residuals for nitrate and 3-NOP treatments from a model where methane yield was the dependent variable, were compared within cow with RCE for methane yield, DMI, VFA, and milk measures. Pearson correlations between individual cow's treatment residuals and cows' RCE were calculated. A positive residual indicates that the cow responded less to the methane-reducing additive than the average cow, and vice versa. Residuals for nitrate and 3-NOP treatments were both positively correlated to RCE for methane yield ($r = 0.30$, $P < 0.001$ and $r = 0.42$, $P < 0.001$) and CO_2/kg DMI ($r = 0.16$, $P < 0.04$ and $r = 0.27$, $P < 0.001$), respectively. Further, residuals for 3-NOP treatment (but not nitrate) correlated positively to milk fat concentration and butyrate molar proportion, and correlated negatively to DMI, all milk production (kg/d) measures, and propionate molar proportion. In conclusion, nitrate and 3-NOP are less efficient reducing methane emission in high-emitting cows, and further 3-NOP was less efficient for cows with RCE for high milk fat concentration and high rumen butyrate molar proportion, and more efficient for cows with RCE for high DMI, high milk production, and high rumen molar propionate proportion.



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144 Recent advances in enteric methane mitigation: insights from a meta-analysis of tannin-containing-diets in small ruminants

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Ruminants play an important role in global food systems, particularly within smallholder farming systems that support over 600 million people globally. However, livestock are significant contributors to global methane emissions, accounting for over 32% of all anthropogenic methane. Under business-as-usual scenarios, these emissions are likely to increase as demand for livestock products rises, driven by a growing population and a projected 40% increase in global per capita income by 2050, which is expected to fuel greater consumption of animal-based products. Addressing this challenge is critical to reducing greenhouse gas (**GHG**) emissions while maintaining livestock productivity. The Low-Methane Forages (**LMF**) project, led by three CGIAR Centers (Alliance Bioversity-CIAT, ICARDA, ILRI), addresses these challenges by promoting Antimethanogenic Compounds (**AMC**)-rich forage legumes, breeding methane-reducing traits into forage crops, and deploying these solutions in livestock systems across the Global South. The project aims to mitigate climate impacts by integrating innovative forages while sustaining ruminant productivity and food security. To support this process, this meta-analysis evaluates the effectiveness of tannin-containing diets in reducing enteric methane emissions in small ruminants, based on a systematic review of peer-reviewed studies published between 2019 and 2023. Boolean search strings containing combinations of the words enteric methane, goat, sheep, small ruminant, condensed tannin, tanniferous forages, and secondary compounds were applied in the Web of Science database. A total of 19 studies were retrieved, including 230 treatment and control mean comparisons for daily CH₄ emissions and 160 comparisons for CH₄ yield per kilogram of dry matter intake (**DMI**). Methane emissions were analyzed by extracting data on daily methane output from the selected studies, which utilized standard respiration chamber techniques, portable gas analyzers, or in vitro fermentation methods, depending on the study. Results demonstrate that tannin-containing diets decrease daily methane emissions and methane yield (g kg DMI⁻¹) by 21.6 ± 2.65% ($P < 0.001$), and 14 ± 2.65% ($P < 0.001$), respectively. Moreover, Productivity, measured as average daily gain (**ADG**), was maintained or slightly improved in the 13 studies reporting ADG, with none showing a decline associated with methane reductions. In addition, treatments integrating tannin-rich roughages demonstrated simultaneous daily methane emissions reductions (-10.5 ± 2.09%, $P < 0.001$). These results support the conclusion that tannin-containing diets reduce methane emissions while maintaining or even increasing DMI. High heterogeneity in the dataset ($I^2 = 95.8$) highlights the variability in tannin efficacy across studies, influenced by factors such as tannin concentration, forage type, animal management practices, and geographic region. Subgroup analyses indicate that dose-dependent effects are critical in determining methane reduction potential, underscoring the importance of balancing tannin concentrations to achieve maximum mitigation while minimizing the risk of potential adverse impacts on feed intake and digestibility. These subgroup analyses revealed that condensed tannins showed inconsistent effects on daily methane emissions, with a mean difference close to zero ($P = 0.44$). While tannin-containing diets promise to reduce methane emissions and maintain productivity, the variability and uncertainty in their efficacy highlight the need for further research to optimize their application in sustainable livestock management.

392 Performance, methane emission, and immune response of dairy cows fed rapeseed, grape marc and combination

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Grape marc, a by-product of wine production, and dietary fat such as rapeseed have been individually used to reduce enteric methane production in ruminants. However, the combined effects of these two dietary strategies on methane emissions, immune response, and rumen microbiome remain unclear. To elucidate this, four lactating Norwegian Red cows were fed four diets in a Latin square design with 10 days of adaptation and 3 days of sample and data collection over 4 periods. The treatments included: control diet (**CON**), diet with 40 g/kg DM of whole cracked rapeseed (**WCR**), diet with 270 g/kg DM of dried grape marc (**GM**), and diet with combination of 40 g/kg DM of WCR and 270 g/kg DM of GM. All diets were formulated to be isonitrogenous and isoenergetic. Methane production was measured using GreenFeed system during each sampling period in 3 consecutive days starting at 0900, 1400, and 2100 h (sampling d 1), 0300, 1100, and 1900 h (sampling d 2), and 0000, and 0600 h (sampling d 3). Adaptive and innate immune response were evaluated *in vitro* using peripheral blood mononuclear cell (**PBMC**) proliferation upon antigen stimulation (concanavalin A and phytohemagglutinin) and whole blood tumor-necrosis factor alpha (**TNF-**) response to lipopolysaccharide stimulation, respectively. Data were subjected to linear mixed effect model, where WCR, GM and their interaction were included as fixed effects. Animal was included as random effect. Dry matter intake of the cows was not affected ($P = 0.52$) by the addition of rapeseed (average DMI of 25 kg/d), but increased ($P = 0.007$) with grape marc by 20%. The WCR and GM treatment had no effect ($P > 0.102$) on milk yield, energy-corrected milk yield, milk fat and milk lactose concentration, but combination of WCR and GM reduced ($P < 0.05$) milk protein concentration from 3.5 to 3.4%. Methane yield (methane production expressed per unit dry matter intake) was not affected ($P = 0.175$) by WCR inclusion (average methane yield of 20 g/kg DMI) but was reduced ($P < 0.001$) by 25% with the addition of GM. No additive effect in methane yield was observed when WCR and GM were included in combination. Additionally, neither the inclusion of WCR nor GM in the diet did affect ($P > 0.118$) *in vitro* PBMC proliferation and whole blood TNF- response. Our findings suggest that while GM alone was effective in reducing methane yield without compromising immune response, the combination with WCR at concentrations used herein did not provide additional benefit. The ongoing analysis on the rumen microbial composition is expected to provide insight on the mechanism behind the observed effect on methane yield.

258 Tropical foliages reduce methane and enhance digestibility in cattle diets

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Enteric methane (**CH₄**) emissions from livestock significantly contribute to global greenhouse gas inventories, particularly in tropical regions where cattle are primarily fed low-quality grasses. This study assessed the effects of incorporating tropical tree foliage of *Bursera simaruba* and *Brosimum alicastrum* into cattle diets on CH₄ emissions, nutrient digestibility, and rumen fermentation parameters. Six crossbred heifers (*Bos indicus* × *Bos taurus*) fitted with rumen cannulas and weighing 377 ± 56.55 kg were allotted to a 3 × 3 replicated Latin square design. The experimental treatments consisted of a control (**CON**) diet with no tree foliage, a diet with 20% *B. simaruba* (**T2**), and a diet with 10% *B. simaruba* + 10% *B. alicastrum* (**T3**), offered as total mixed rations (**TMR**). The experiment was conducted in metabolic crates and in open-circuit respiration chambers for CH₄ measurements. Daily dry matter intake (**DMI**) and apparent nutrient digestibility were determined. Rumen fermentation parameters, including volatile fatty acid (**VFA**) profiles, were measured through rumen fluid sampling and high-performance liquid chromatography (**HPLC**). Response variables were subjected to analysis of variance (**ANOVA**) using the weight of cattle as a covariate and the post hoc Tukey test was used to check for significant differences in the means ($p \leq 0.05$). The inclusion of tree foliages did not significantly affect DMI or nutrient intake (DM: 9.69 kg/day; CP: 0.96 kg/day; NDF: 6.09 kg/day). However, apparent nutrient digestibility decreased in T2 compared to CON, attributed to the high condensed tannin (**CT**) content in *B. simaruba*. In contrast, T3 showed improved digestibility of DM (60.2%), OM (58.3%), and NDF (46.9%) compared to CON. Methane emissions were significantly reduced with both treatments: T2 achieved a 21.2% reduction (189 g CH₄/day) and T3 a 10.3% reduction (215 g CH₄/day) relative to CON (240 g CH₄/day). Methane production per kg of DMI was also reduced, particularly with T2; 19.51 g CH₄/kg of DMI and 22.75 g CH₄/kg of DMI for T3 compared to 24.64 g CH₄/kg of DMI for CON. Similar molar proportions of acetic, isobutyric and butyric acids were registered in rumen liquor between treatments, as well as pH values. Total VFA concentrations were highest in T3 (83.7 mmol/L), while the acetic:propionic acid ratio slightly increased across experimental diets.

The experiment highlights the potential of tropical forages containing CT to mitigate CH₄ emissions in tropical livestock production systems. While *B. simaruba* was effective in reducing CH₄ emissions, its high tannin content negatively affected nutrient digestibility. Conversely, the combination of *B. simaruba* and *B. alicastrum* balanced CH₄ mitigation with improved digestibility, suggesting a synergistic effect. These findings underscore the importance of strategic forage combinations to optimize both environmental and production outcomes. Tropical tree foliages represent a sustainable option for CH₄ mitigation in cattle diets, particularly in resource-limited regions. Future research should address seasonal variability in foliage availability and CT concentrations to develop practical applications.

111 Bioactive forages: a promising path to mitigate enteric methane emissions in the livestock sector

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Dietary modification is deemed to be one of the most effective and pragmatic mitigation strategies to reduce methane (**CH₄**) from enteric fermentation, as it not only influences rumen fermentation patterns and their end-products but also has the potential to concomitantly enhance animal productivity. The inclusion of bioactive forages is particularly promising in pasture-based livestock systems where they can achieve these goals, and simultaneously reduce food-feed competition compared to concentrate-rich rations with antimethanogenic feed additives. Therefore, we conducted a greenhouse experiment to screen eight temperate forage species with different concentrations of tannins and flavonoids as bioactive compounds. The species were analyzed for their agronomic potential, plant specialized metabolite (**PSM**) profile, as well as their in vitro antimethanogenic potential. In general, the largest reductions in CH₄ were observed from the tannin-rich forages (2 to 48%), sulla (*Hedysarum coronarium*), big trefoil (*Lotus pedunculatus*), birdsfoot trefoil (*Lotus corniculatus*), sainfoin (*Onobrychis viciifolia*) and salad burnet (*Sanguisorba minor*), in comparison to lucerne (*Medicago sativa*) as a tannin free control. However, this reduction was also accompanied by a reduction in total gas production (**GP**, -2 to 42%) in some species, indicating a negative effect on rumen degradability. The variation in the antimethanogenic activity at the species level was found to be significantly linked with the variation in their tannin composition, particularly influenced by tannin (proanthocyanidins (**PA**s) and hydrolysable tannins (**HT**s)) concentration and PA polymer size. Additionally, forage quality parameters played a significant role, highlighting the need to consider multiple interacting factors to understand bioactivity comprehensively. Based on this, a follow-on study was conducted, using big trefoil (PA-rich) and salad burnet (HT-rich) as model species, as they had the lowest variability in terms of their antimethanogenic activity and tannin composition across harvests. Of these, 200 individual plants from each species have been screened for their PSM profile and were subsequently genotyped via next generation sequencing. Subsequently, all plants will be analyzed for in vitro gas and CH₄ production, and fermentation characteristics. The data is expected to be ready in early 2025. In conclusion, we have previously identified the antimethanogenic potential of tannins from these species to suppress CH₄ in ruminants in vitro, but the potential is affected by covariables, such as the forage quality. Our next experiments will enable us to identify how much of this variation is genetically determined, and to which degree these forages can be improved to provide strong and reliable antimethanogenic effects without reducing digestibility.

71 Low methane phenotype of beef feedlot cattle changes throughout production and depends on diet

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A better understanding of the variability in methane (**CH₄**; g/day) emissions from beef cattle throughout production is necessary to implement mitigation programs. One hundred Angus steers were fed diets that included either barley silage (**BS**) or corn silage (**CS**) throughout a backgrounding (**BG**) and finishing (**FN**) phase feedlot study. The objectives were to characterize the variability in methane yield (**CH₄Y**; g/kg dry matter intake) among and within steer during BG and FN phases of production, assess whether the variability in CH₄Y differs by silage type, examine the stability of CH₄Y ranking (Low, Intermediate, or High) of steers within and across phases, and evaluate relationships between range of CH₄Y, CH₄ production, and feeding behaviour. Cattle were fed a backgrounding diet (70% silage, 25% barley, 5% mineral supplement; on a DM basis) for 121 d and a finishing diet (15% silage, 80% barley, 5% mineral supplement; on a DM basis) for 158 d, with each phase comprised of two measurement periods. Enteric CH₄ was measured using GreenFeed emissions monitoring systems (C-Lock Inc., USA) for 2 weeks/period in backgrounding and 2-3 weeks/period in finishing. Steers were ranked based on their CH₄Y (**MYR**) in each period and phase and categorized as Low (<0.5 SD from mean), Intermediate (within ± 0.5 SD of mean), or High (>0.5 SD of mean) emitters. Statistical analysis was performed within a period or phase using PROC GLIMMIX of SAS as a mixed model with fixed effects of MYR, silage, MYR $\times$ silage, and period or phase. Steer was the experimental unit, and the random effect to account for individual variability. Rankings of individuals were compared across periods and phases to determine whether rankings changed over time. The CH₄Y of steers ranked Low in period 1 were not different from Intermediate steers in period 2 of BG ($P < 0.01$). Similarly, steers ranked as Low in BG overall were not different from those ranked as Intermediate in FN for CH₄Y ($P < 0.01$). However, during finishing, Low and Intermediate steers differed in period 2, whether using period 1 or 2 ranking ($P < 0.001$). Steers were more likely to change MYR during backgrounding (56%) and from backgrounding to finishing (42%), compared to during finishing (20%). During backgrounding, 14% of BS and 71% of CS steers ranked as Low in period 1 changed rank in period 2. When steers were ranked as Low during backgrounding, 43% (BS) and 57% (CS) had a different MYR in finishing. Pearson correlation coefficients revealed a moderate to strong positive relationship between the CH₄Y range and CH₄ and CH₄Y during finishing for both silage sources ($P < 0.05$). For CS steers, as the CH₄Y range increased, feed intake decreased ($P < 0.001$; $r = -0.61$). In conclusion, the variability in CH₄Y was present throughout backgrounding and finishing, and MYR was most likely to change within backgrounding and from one phase to the next. Therefore, these results suggest that selecting steers for their MYR based on a short-term measurement may not reflect lifetime MYR within and across phases and diets.

163 Chemical pre treatments of low unconventional roughage to moderate in rumen microbial fermentation

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The purpose of this study was to investigate the effects of treating olive (*Olea europaea*) leaves with sodium hydroxide (**NaOH**), urea (4% for 40 d), or polyethylene glycol (**PEG**) supplementation (100 mg/g DM) on their nutrient composition, in vitro gas production, greenhouse gas emissions, and fermentation profile. Incubations were performed in rumen liquid and done in three separated runs. Rumen liquid was issued from three slaughtered adult sheep in slaughtering house in Cairo. A sample of 1.0 g of *O. europaea* (untreated or treated with NaOH, or urea or supplemented or not with PEG) was placed into fiber bags and then transferred to 250 mL incubation Ankom bottles. Pressure readings were recorded each 10 min for 48 h of incubation, cumulative pressure values were then calculated. At the end of incubations, 5 mL of gas production were collected from the headspace and analysed to measure the concentration of methane (CH₄) and carbon dioxide (CO₂). The results showed that NaOH-treated leaves had the lowest organic matter and fiber fractions, while urea-treated olive leaves exhibited the highest crude protein content ($P < 0.001$). Asymptotic gas production was significantly higher ($P = 0.015$) in urea-treated olive leaves compared to NaOH-treated leaves. CH₄ production was highest ($P < 0.05$) in urea-treated leaves and lowest in NaOH-treated leaves. During incubation, urea-treated leaves produced the highest CO₂ per gram of degradable neutral detergent fiber ($P = 0.015$). Degradability parameters indicated that PEG supplementation led to the highest DM and acid detergent fiber degradabilities ($P < 0.05$) compared to the untreated and NaOH-treated leaves. Urea-treated leaves produced the most acetic acid ($P = 0.016$), comparable to the control but significantly higher than PEG-supplemented and NaOH-treated leaves. Butyric acid levels were higher ($P = 0.011$) in NaOH-treated and PEG-supplemented leaves than in the control and urea-treated leaves. Metabolizable energy (**ME**), calculated as $2.20 + 0.136 \text{ GP} + 0.057 \text{ CP}$, was significantly higher ($P = 0.013$) in urea-treated leaves than in untreated and NaOH-treated leaves, with the latter producing the lowest ME. PEG supplementation resulted in significantly higher microbial protein (**MCP**) production ($P = 0.023$) compared to NaOH-treated and untreated leaves, while MCP level in the urea-treated group was comparable to all other treatments. In conclusion, PEG supplementation was the most effective treatment for olive leaves degradability and MCP, with urea treatment being the second option. From an environmental perspective, NaOH treatment was the most effective for reducing CH₄ and CO₂ emissions during the fermentation of olive leaves. By addressing the dual challenges of waste management and resource efficiency, this study insights into the role of olive leaves in reducing the ecological footprint of livestock production in Mediterranean area under climatic chngement context.

153 Nutritional composition and *in vitro* methane production of tropical grass-shrub mixtures in the Peruvian Amazon

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Silvopastoral systems represent a sustainable and high-potential alternative for the development of livestock in the Peruvian Amazon. Shrub species such as *Leucaena leucocephala* (leucaena) and *Gliricidia sepium* (matarratón) have been widely studied assessing nutritional composition and their effect on gas and methane production. However, there are few studies on other species such as *Morus alba* (mulberry) and *Cratylia argentea* (veranera), although they are considered shrubs with great potential for use in silvopastoral systems. Therefore, the aim of the present research was to evaluate the nutritional composition and *in vitro* methane production of mixtures of a conventional grass (*Urochloa brizantha*) with *M. alba*, and *C. argentea* adapted to Peruvian tropical conditions.

Tropical forages (*A. morus*, *C. argentea*, and *U. brizantha*) were collected during the rainy season in the region of San Martín, Peru. Forages were evaluated independently and in mixtures with a single ratio (70% grass plus 30% shrub legumes) in the Laboratorio de Evaluación Nutricional de alimentos (LENA), Universidad Nacional Agraria La Molina. Crude protein, neutral detergent fiber (FDN), and ash content were quantified from a total of 5 treatments (Trt1 = *U. brizantha* 70 % + *M. alba* 30%; Trt2 = *U. brizantha* 70 % + *C. argentea* 30%; Trt3 = *U. brizantha* 100 %; Trt4 = *M. alba* 100%; Trt5 = *C. argentea* 100%). Subsequently, the previous treatments were incubated in triplicate at three different times at 39°C with rumen liquor and artificial saliva inside gas release bottles. After incubation, at the following 3, 6, 12, 24, 36, 48, 72 and 96 hours the volume of the total gas produced was measured, while at 12, 24 and 48 hours a gas sample was taken to quantify the concentration of methane by gas chromatography and then the *in vitro* digestibility of the dry matter was measured, based on a relationship between what was incubated and the content obtained at the end of incubation at these times. A randomized complete design and a randomized complete block design were used for the analysis of the nutritional value, production of methane, and gas from the treatment. Statistical model and program to be used will be reported when data are available.

Experimental trials are ongoing, but it is expected that the inclusion of 30% shrub species will make a significant contribution to improving the quality of animal feed and reducing methane production. The research will be completed, reported, and shared at the time of the conference. This study seeks to contribute to the development of more sustainable and efficient livestock production practices in the Amazon region, offering alternatives that benefit both producers and the environment.

75 Exploring Egyptian forage legumes for ruminal fermentation and methane mitigation

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The productivity of ruminant livestock in tropical and subtropical regions is often limited by inadequate nutrition, characterized by low-quality forage and feed shortages. Addressing these challenges requires exploring alternative feed resources with high nutritional value, such as forage legumes. Forage legumes offer superior degradable and undegradable protein levels, and their natural phenolic compounds, particularly condensed tannins (**CTs**), can improve ruminal fermentation, reduce ammonia production, and enhance amino acid absorption while mitigating methane (**CH₄**) emissions. This study investigated the potential of Egyptian forage tree legumes, *Acacia saligna*, and *Leucaena leucocephala*, as alternative feed sources compared to the standard grass hay, berseem clover (*Trifolium alexandrinum*). The hypothesis was that these legumes would enhance ruminal fermentation and reduce methanogenesis, improving nitrogen utilization.

The consumable parts of *Acacia* and *Leucaena* were harvested from 35 trees in Borg El Arab, Alexandria, Egypt, dried, milled, and chemically analyzed. Phenolic compounds were quantified using high-performance liquid chromatography. An *in vitro* gas production assay assessed tannin bioactivity over 24 hours, with and without polyethylene glycol (**PEG**), to isolate tannin effects. Methane and carbon dioxide (**CO₂**) concentrations were analyzed via gas chromatography, while short-chain fatty acids (**SCFAs**), truly degraded organic matter (**TDOM**), ammonia nitrogen (**NH₃-N**), and pH were evaluated using standard procedures. Data were analyzed using the PROC GLM) of SAS.

The results showed that *Acacia* has the superiority of tannins bioassay calculated as the percentage of cumulative gas production for 24 h when compared to *Leucaena*. The TDOM of *Leucaena* was higher ($P < 0.001$) than other forages, even with or without PEG addition, which may indicate the presence of another factor that enhances fermentation and degradability other than the low content of CTs. The higher of pH for *Acacia* and *Leucaena* without PEG addition could be attributed to the presence of tannins while the increase in **NH₃-N** with PEG may be related to low tannins content in both Berseem and *Leucaena*, which had higher crude protein (**CP**) digestibility. Notably, *Leucaena* increased propionate production ($P = 0.014$), indicating its potential for **CH₄** reduction by redirecting hydrogen from methanogenesis to propionate synthesis.

In conclusion, *Leucaena leucocephala* demonstrates significant potential as a promising alternative forage resource in the Mediterranean region. Its ability to increase bypass protein and have some advantages, such as reducing methanogenesis, and improving nitrogen utilization positions it as a sustainable solution to the nutritional challenges ruminants face in tropical and subtropical regions. Future studies should further investigate its integration into livestock feeding systems to maximize its environmental and economic benefits.

179 Life cycle assessment of drought-tolerant grasses for sustainable dairy farming

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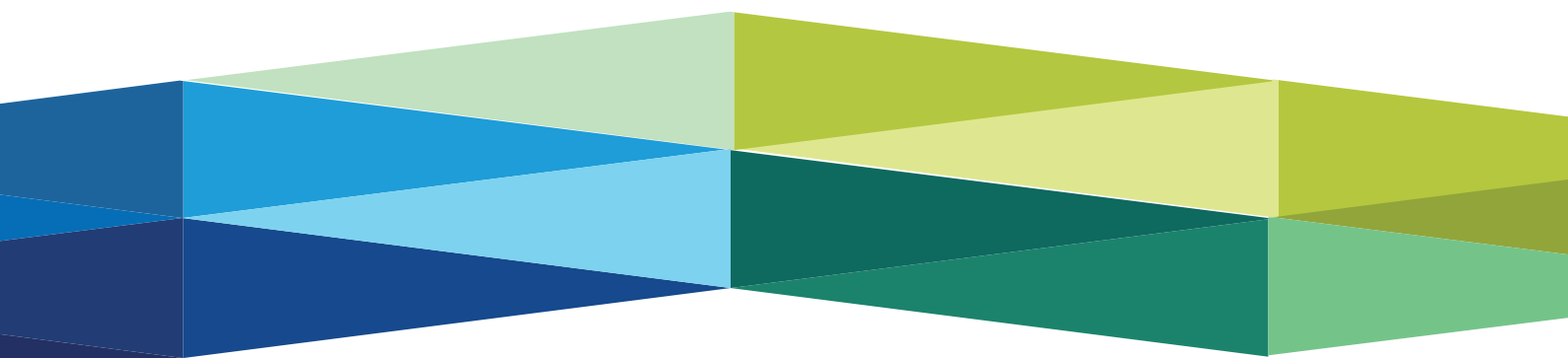
Dairy farming in Flanders faces mounting challenges from climate change, notably increased droughts. Traditional forages like perennial ryegrass are highly vulnerable to drought, leading to significant yield losses and environmental impacts. This study uses Life Cycle Assessment (LCA) to evaluate the implications of adopting drought-tolerant grasses, such as tall fescue, and integrating red clover into dairy cow diets. The analysis employed a model farm with 100 dairy cows and 75 youngstock under eight scenarios varying forage type, red clover inclusion, and weather conditions in wet (2021, 2023) and dry (2022) years. The cradle-to-farm-gate boundary encompassed feed production, manure management, and enteric methane emissions, with results expressed per kilogram of fat and protein corrected milk and per cow per day. Replacing perennial ryegrass with tall fescue increased forage yields, especially in dry conditions, with tall fescue producing 2–4 tons more per hectare. This improvement reduced land use and the environmental impacts from feed production per hectare. However, tall fescue diets had lower digestibility, leading to higher enteric methane and manure emissions and a greater climate impact per kilogram of milk. For instance, in a dry year, tall fescue diets had a climate impact of 1.09 kg CO₂-eq per kilogram of milk compared to 1.06 for perennial ryegrass diets. Furthermore, fescue diets relied more on soy, significantly contributing to the environmental footprint of feed purchases, which accounted for up to 40% of the total climate impact in some scenarios. The inclusion of red clover up to 3.55 kg dry matter per day or 14.9 % of the total ration, enhanced sustainability in both perennial ryegrass and tall fescue systems. Red clover reduced fertilizer and diesel needs, lowering emissions from feed production. Its inclusion also decreased soy reliance and improved tall fescue digestibility by 1.3%, partially offsetting negative impacts on methane and manure emissions, with reductions up to 3.8% per unit of milk. In perennial ryegrass diets, red clover lowered overall environmental impacts, reducing climate impact per kilogram of milk by up to 1.3% compared to diets without it. Drought conditions increased the environmental impacts across all forage systems. Perennial ryegrass yields fell from 15.5 to 10.0 tons per hectare in 2022, necessitating extra maize purchases, while tall fescue yields dropped only from 17.5 to 14.0 tons per hectare. This resilience resulted in smaller climate impact increases during drought for tall fescue. This underscores its potential to improve the robustness of forage systems under climate stress. The study highlights trade-offs between higher yields and lower nutritional value when switching to tall fescue and emphasizes red clover's complementary benefits from a LCA perspective. Limitations include reliance on a model farm and the inability to fully isolate dietary effects. Future research should expand to diverse farming systems and explore the long-term impacts of these dietary changes to provide a holistic understanding of the environmental performance of drought-tolerant forages.



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1.4 Forage-based strategies for reducing enteric methane emissions



470 Effect of weaning age on lifetime methane emissions from growing heifers in a grazing system

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Later weaning of calves can increase average daily gain (**ADG**) in early life, while early weaning promotes solid feed intake and accelerates morpho-physiological development of the rumen. Both could influence methane emissions and animal production later in life. The objective was to determine the effect of calf weaning age on methane (**CH₄**) emissions measured three times after weaning. Hereford×Friesian heifer calves (n=72) born in autumn 2022 received 1 kg milk replacer per day and *ad libitum* pasture. The calves were then weaned at either 10 (EARLY) or 20 weeks (LATE) of age and kept on ryegrass-based pasture for the remainder of their lives. Methane and carbon dioxide (**CO₂**) emissions were measured three times (measurement periods), when the heifers were on average 10, 12, and 22 months of age, using large ruminant GreenFeed emissions monitoring units in the field. Dry matter intake (**DMI**) was estimated based on body weight and average daily gain, and the residual CH₄ was estimated as the residual error of the CH₄ emissions as a function of the CO₂ emissions. The data were analysed using a linear mixed model in R (version 4.3.0), considering weaning age, measurement period, and their interaction as fixed effects.

Methane production (g/d) and yield (g/kg DMI) were both 4% greater in LATE vs EARLY weaned heifers (P = 0.015). There was no interaction between weaning age and period for gas emissions parameters. The residual CH₄ (P = 0.079), CH₄ to CO₂ ratio (P = 0.098), and CO₂ emissions (P = 0.093) trended to be lower in EARLY weaned heifers. DMI was similar for EARLY and LATE weaned heifers (P = 0.893), suggesting that emissions differences were not related to feed intake. When extrapolated from 5 to 23 months of age (slaughter age), the ADG was 6% higher (44 g/d; increase from 160 to 551 kg in EARLY and 179 to 548 kg in LATE), and CH₄ intensity (g/kg ADG) was 12% lower in EARLY weaned heifers. These results suggest that pre-weaning management of growing heifers can influence CH₄ emissions later in life, both in absolute terms and per unit of animal product.

264 Impact of tropical cultivated forages on enteric methane emissions in Zebu cattle in West Africa

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Cultivated forages are now being popularized among ruminant farmers to improve animal feeding conditions. However, their impact on enteric methane (**eCH₄**) emissions remains poorly understood. This study aimed to compare the eCH₄ emissions from cultivated forage-fed animals with those from animals fed exclusively rangeland fodder (**RF**). The three most cultivated and used grasses hays by cattle farmers in West Africa, namely *Brachiaria ruziziensis*, *Andropogon gayanus*, and *Panicum maximum*, were harvested at maturity stage and offered each to ten steers (27.7±4.6 months, 143.7±15.7 kg initially) at 3.2 and 2.3% live weight (**LW**) dry matter (**DM**) basis respectively. The control diet (RF) was distributed at the same quantities. The experiment lasted three weeks for each forage and feeding level, and included two weeks of adaptation and one week of individual data collection. Those data are voluntary intake (refusal collection), apparent digestibility (total feces collection), and eCH₄ (GreenFeed® system). The type of forage influenced the chemical composition, intake and digestibility of the diet, and the amounts of eCH₄ emitted by the animals. Only the *P. maximum* diet had a better nutritional quality, thus higher intake by animals, than RF (*P*<0.001). The latter had an intermediate apparent digestibility between that of *P. maximum* and those of the other forages (*P*<0.05). The lowest eCH₄ production (*P*<0.001) and the highest eCH₄ yield (*P* = 0.4868) were recorded in animals fed *A. gayanus* hay. Per unit of digestible matter intake, animals fed RF showed an intermediate yield between that of those fed *A. gayanus* and other grasses. Reducing feed supply from 3.2 to 2.3% LW did not affect intake, apparent digestibility or eCH₄ emissions. At maturity, the eCH₄ yield potential of all of the cultivated grasses investigated in this study was similar to that of RF. However, *P. maximum* may be preferred over other grasses due to its low eCH₄ yield per unit of digestible matter intake. Measuring eCH₄ emissions induced by these grasses at different stages of growth will make it possible to specify their optimum stage of use in the context of environmental sustainability.

76 Mid-lactation dairy cows have lower methane emissions when grazing pasture containing plantain (*Plantago lanceolata* L.)

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In Ireland, enteric methane emissions account for approximately 65% of agricultural-related greenhouse gas emissions. Plantain (*Plantago lanceolata* L.) has been identified as a forage to mitigate nitrogen loss from dairy systems and has shown potential to reduce enteric methane emissions due to its lower digestibility and plant secondary compounds. However, no data currently exists in the literature on methane emissions of lactating dairy cows grazing perennial ryegrass (*Lolium perenne* L.)-white clover (*Trifolium repens* L.)-plantain pasture. This study aimed to quantify methane emissions of dairy cows grazing perennial ryegrass-white clover pasture with or without plantain. It was hypothesised that cows grazing pasture containing plantain would have lower methane emissions.

This complete block design utilised Holstein × Friesian dairy cows, with two pasture treatments; 1) perennial ryegrass (77%)-white clover (16%) (**GC**; n = 12), and 2) perennial ryegrass (50%)-white clover (11%)-plantain (33%) (**GCP**; n = 13). Gaseous emissions were measured over 41 days using two GreenFeed units. Cows were on average 135 ± 17 days in milk at the beginning of the measurement period. Cows were offered fresh pasture (15 – 17 kg DM/cow per day) and received a pelleted concentrate feed (2.71 kg DM/cow per day) through two split allocations (0730 and 1500 hours) during milking.

Data were screened for outliers and analysed using SAS Studio® (version 3.81). Individual gas measurements for each treatment were adjusted for parity, GreenFeed unit, days in milk, and measurement hour using PROC MIXED. Adjusted values were then analysed using PROC MIXED with the fixed effect of treatment. Covariates included cow economic breeding index, body condition score, metabolic live weight, and total number of measurements. Cow was included as a random effect.

There was no effect of treatment on milk yield (mean = 24.1 kg/day, SEM = 0.73; *P* = 0.85) or milk solids yield (mean 1.95 kg/day, SEM = 0.051; *P* = 0.13). Treatment did not affect the average daily visits (mean = 1.77 visits/day, SEM = 0.156; *P* = 0.66), visit duration (mean = 238 seconds, SEM = 5.3; *P* = 0.93), or the quantity of concentrate offered by the GreenFeed unit (mean = 0.52 kg DM/cow per day, SEM = 0.043; *P* = 0.54). Cows grazing GCP had lower methane emissions (327 vs. 360 g/day, SEM = 8.8; *P* = 0.01), methane per kg of milk yield (13.4 vs. 15.0 g/kg of milk yield, SEM = 0.47; *P* = 0.03), and carbon dioxide emissions (11.3 vs. 13.2 kg/day, SEM = 0.17; *P* < 0.001) compared to cows grazing GC. There was no effect of treatment on methane intensity (mean = 175 g/kg of milk solids, SEM = 5.4; *P* = 0.31) or hydrogen emissions (mean = 2.17 g/day, SEM = 0.108; *P* = 0.29).

Including plantain in perennial ryegrass-white clover pasture, offered to mid-lactation dairy cows, reduced methane emissions without negatively affecting milk production. Further investigation should be conducted during the early and late lactation periods.

385 Efficacy of bromoform supplementation to inhibit methane emissions from cattle grazing low and high-quality pasture

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Bromoform (**CHBr₃**) has been recognised as a potent methane (**CH₄**) inhibitor in ruminants by blocking the action of methyl-coenzyme M reductase and methyltransferase enzymes during CH₄ formation in the rumen. However, administering supplements to grazing cattle can be challenging due to the reliance on voluntary intake, predominately influenced by environmental conditions, pasture availability, and animal behaviour. This study examined the effectiveness of pellet supplementation containing CHBr₃ for lowering CH₄ production (g/day) in backgrounding steers when grazing either low- or high-quality pasture. Seventy-two Brangus steers, aged 18 months and with a mean liveweight (**LW**) of 324 ± 22.8 kg, were randomly allocated to four paddocks (18 head/paddock) of 10 ha each. Within group paddocks, steers grazed for 63 days on high-quality pasture, followed by 65 days on low-quality pasture, with oats and ryegrass as the primary species. A supplementation yard (25 x 25m) comprising four zones was situated within each paddock and equipped with a water source (zone 1), a 3-way auto drafter-linked walkover weigh leading to zones 2, 3 and 4, each fitted with a custom-built UHF-RFID attached to a supplement feeder. To measure individual CH₄ production, a GreenFeed system was placed in each paddock, delivering a pellet at 1 kg/head/day to entice steers visiting the unit for continuous CH₄ spot sampling. Forage quality differed between high and low-quality pastures in metabolisable energy (12.6 MJ/kg DM vs 9.1 MJ/kg DM), dry matter digestibility (82.8% vs 62.4%), NDF (44.6% vs 58.9%), ADF (23.2% vs 31.4%), crude fat (2.5% vs 1.7%), and crude protein (5.2% vs 3.8%). Within each group paddock, steers (*n* = 18) were split into three subgroups: control (**C**; *n* = 6) and two treatment groups (**T1**; *n* = 6 and **T2**; *n* = 6). The control group received canola-based pellets, while the treatment groups were offered canola-based pellets containing natural (T1) and synthetic (T2) CHBr₃ dosed at 280 mg/kg DM. Steers from zone 1 were auto-drafted based on individual RFID eartags to zones 2, 3 or 4 to access their respective supplement at 1.67 kg/head/day before returning to the paddock. Over the 63-day period, T1 and T2 reduced CH₄ production significantly by 18.7% and 13.8%, respectively, from 165.9 to 134.8 and 143.0 g/day (*P* < 0.001), with supplement intake being 1.12, 0.87, 0.78 kg/day, respectively, and no significant effect on LW gain (1.5, 1.4, and 1.4 kg/day; *P* > 0.05). When grazing low-quality pasture, CH₄ production for T1 and T2 was significantly lower by 74.6% (42.2 g/day) and 70.19% (45.9 g/day) relative to the control group (153.6 g/day) (*P* < 0.001), with supplement intake of 1.6, 1.5, and 1.5 kg/day and LW gain of 0.8, 0.7, and 0.7 kg/day (*P* > 0.05). No significant difference in CH₄ production was observed between T1 and T2 across both pasture periods (*P* > 0.05). Although CH₄ production of cattle supplemented with CHBr₃ was clearly suppressed, the results accentuated the greater efficacy of CHBr₃ in reducing emissions under low-quality forage conditions.

397 Effects of dose and frequency of seaweed supplementation on enteric methane mitigation in grazing sheep

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Red seaweed (*Asparagopsis taxiformis*), whose main bioactive compound is bromoform, has demonstrated up to 80% mitigation of enteric methane (CH_4) emissions in sheep fed a total mixed ration (TMR). However, most global ruminant production occurs in extensive grazing systems, where TMRs are not feasible. This necessitates further exploration of the effectiveness of this feed additive when delivered as a supplement. This study conducted two experiments to quantify the effects of increasing dose and frequency of *Asparagopsis taxiformis* extract supplementation in grazing sheep at the University of New England's Smart Farm, Australia. In Experiment 1 (dose effect), 80 Merino hoggets (initial live weight: 54.5 kg) were used in a completely randomized design with four increasing concentrations of bromoform (mg/kg DM) in supplementary pellets: 0 (Control), 159 (T2), 319 (T3), and 478 (T4). GreenFeeds (C-Lock, South Dakota, USA) were used to deliver the supplementary pellets and measure CH_4 emissions. Each sheep was allowed access to up to 150 g of pellets daily, divided into eight equal meals, four hours apart. Each treatment group (20 hoggets) grazed on 2.1 ha paddocks dominated by native pastures, with each paddock fitted with a GreenFeed unit. The treatment groups were rotated weekly among the four paddocks used to eliminate paddock and Greenfeed unit effect. The sheep were trained to use the GreenFeed over 28 days, adapted to the experimental diets for 14 days, and CH_4 emissions were measured over 28 days. In Experiment 2 (frequency effect), the same animals were used to test four different supplementation frequencies, following the same experimental layout as in Experiment 1: supplemented with untreated pellets once daily (Control), and supplementation with seaweed-treated pellets either twice daily (AM/PM; T6), once daily (T7), or once every second day (T8). Supplementary pellets were formulated to deliver 30 mg of bromoform per sheep daily, regardless of supplementation frequency. A 6-month washout period was allowed between experiment 1 and 2. In both experiments, the supplementary pellets were prepared by mixing ruminant concentrate pellets with either canola oil (control) or canola oil infused with *Asparagopsis taxiformis* extract (SeaFeed™, SeaForest, Australia). Sheep were weighed weekly throughout the experiments. Regression analysis was performed to assess the relationship between dietary bromoform intake and supplementation frequency against CH_4 mitigation. In Experiment 1, CH_4 mitigation increased linearly with bromoform intake ($R^2 = 0.99$): 30% (T2), 46% (T3), and 63% (T4) reductions compared to the control (38.7 g CH_4 /day), with no evidence of plateauing. In Experiment 2, a linear effect was also observed ($R^2 = 0.59$), with CH_4 emissions reduced by 35.2% (twice daily), 14.8% (once daily), and 18.5% (once every second day) compared to the control (44.7 g CH_4 /day). *Asparagopsis taxiformis* supplementation did not affect weight gain ($P > 0.05$). On average, sheep visited the GreenFeeds four times daily, with 79% of the flock successfully trained to use the units. Our findings show that increasing dose of *Asparagopsis taxiformis* supplementation enhances its methane mitigation, with at least twice a day supplementation needed in grazing conditions for higher activity.

253 Effect of grazing multispecies swards on performance and methane emissions of finishing ewe lambs

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Grass-based systems are fundamental to the sustainability and production of the ruminant livestock sector. The simplification of grass seed mixtures has resulted in monoculture grazing systems, which are heavily dependent on high inputs of nitrogen fertilisers, increasing the risk of air and water pollution, greenhouse gas emissions and biodiversity loss. In grazing swards, the inclusion of legume and/or herbs has been shown not only to reduce the dependency on nitrogen input whilst increasing animal production efficiency, but also to mitigate greenhouse gas emissions. However, there is limited evidence of the effects of grazing multispecies swards on performance and methane emissions of finishing lambs and no evidence of predicting grazed grass intake whilst grazing these contrasting sward types. This study assessed the effect of three sward compositions on performance and methane emissions of growing lambs during the mid to late grazing season. Sixty crossbred ewe lambs were allocated to three grazing groups (20 lambs/group), balanced by BW and date of birth. Grazing swards: perennial ryegrass (**PRG**); PRG/white clover (**R/WC**); and multispecies sward (**MSS**; PRG, WC, red clover, timothy, plantain, chicory and trefoil). Lambs were rotationally grazed in a weekly basis for nine weeks without concentrate supplement. Herbage biomass, before and after grazing, of each sward was estimated using the rising platometer and quadrat clips (0.25m²). Prior to grazing, each sward was sampled for NIRS and wet chemistry analyses. Within each group, individual lamb herbage intake (kgDM/d) was calculated using the group measured grazed herbage intake (kgDM/d) multiplied by the proportion of individual sheep ME requirement (**MEreq**) over the sum of group total MEreq. The individual sheep MEreq was predicted as a sum of ME for: maintenance, live weight gain and grazing allowance. Methane emissions were measured from 12 lambs/treatment in a zero-grazing study for 3 weeks using a Greenfeed (C-Lock Inc.), minimal concentrate supplementation. Diet composition during zero-grazing for *PRG*: ME=9.41MJ/kgDM, CP=12.2%, NDF=60.6%; *R/WC*: ME=9.81MJ/kgDM, CP=16.5%, NDF=55.2%; *MSS*: ME=9.51MJ/kgDM, CP=15.1%, NDF=51.5%; *Concentrate*: ME=13.0MJ/kgDM, CP=16.9%, NDF=27.3%. Response variables were analysed using a linear mixed model via REML, with grazing sward as the fixed effect and lamb ID as a random effect. Pairwise differences between treatments were examined using the Tukey's test ($P \leq 0.050$). After 9 weeks of grazing, lambs in the MSS showed $\geq 30\%$ higher ($P < 0.001$) average daily gain (g/d) and $\geq 16.2\%$ better ($P = 0.032$) total period gain (kg/period) than their PRG or R/WC counterparts. Estimated grazed herbage dry matter intake (DMI, kg/d) in MSS and PRG lambs was 17% and 20% higher ($P < 0.001$) than in R/WC lambs. The output in the zero-grazing measurement showed that MSS lambs produced 12.5% less methane (g/d, $P = 0.034$) than PRG lambs. The MSS lambs had 15.9% and 12.7% lower methane intensity (g/kg live weight gain) than PRG and R/WC lambs, respectively. Grazing ewe lambs in multispecies swards is a promising practice to improve the animals' weight gain without comprising DMI, whilst reducing methane emissions.

50 Evaluation of methane emissions from grazing sheep in different regions of Mongolia

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Methane emissions from livestock are a significant contributor to greenhouse gas emissions, with diet composition playing a crucial role in determining emission levels. Mongolia, a landlocked country in the central region of Northeast Asia (87°41'–119°56'E, 41°35'–52°09'N), has a population of 3.5 million and spans 1.56 million km². With its vast rangelands, the country relies heavily on extensive livestock grazing, which is a major component of its agricultural sector. Due to Mongolia's diverse agro-ecological regions, variations in vegetation significantly influence livestock production potential. The agriculture sector accounts for 51.97% of Mongolia's total greenhouse gas emissions, and the country's Nationally Determined Contribution (**NDC**) aims to reduce emissions by 22.7–27.2% by 2030, with methane mitigation from livestock playing a key role in achieving this target. This study investigated the relationship between methane emissions from Mongolian sheep and the chemical composition, including phenolic compounds (tannins), of pasture plants from different ecological regions of Mongolia. Accurate estimation of greenhouse gas emissions using country-specific emission factors (**EFs**) is essential for effective mitigation strategies. Pastures from the mountain meadow, mountain steppe, and Gobi regions were analyzed for their impact on methane production. Four replicate plant samples were collected from 1 m² plots in each region to determine their chemical composition and total phenolic content. In vitro digestibility and gas production were assessed using a split-plot experimental design, while methane emissions were quantified through in vitro gas production techniques and gas chromatography. Results showed that mountain steppe pasture had an organic matter (**OM**) content of 83.6%, crude protein (**CP**) of 11.2%, and organic matter digestibility of 63.5%. In contrast, mountain meadow pasture contained 78% OM, 11.2% CP, and had a lower organic matter digestibility of 45.5%. Methane production was significantly higher from mountain meadow pasture compared to mountain steppe pasture when fermented in rumen inocula from sheep (40.1 vs. 17.92 mL/g DM, respectively). These findings highlight that fiber-rich pasture plants from the mountain meadow region contribute to higher methane emissions than those from the mountain steppe and Gobi regions. The results emphasize the need for country-specific EFs to improve national enteric methane emission estimates and inform mitigation strategies. Overall, this study underscores the importance of pasture composition in developing sustainable and environmentally friendly livestock production systems.

447 Defining new phenotypes for forage improvement based on rumen function and greenhouse gas (GHG) emissions

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Climate change has a significant impact on millions of livestock farmers, herders and their families across Africa and Asia. They face the double burden of needing to decrease environmental and climate footprints while producing more milk and meat and being negatively impacted by climate change impacts, such as heat stress, and increasing frequency and intensity of droughts and floods. There is an urgent need for modern approaches for transitioning low- and middle-income countries (LMICs) to climate smart and sustainable food systems.

Using a diverse set of Napier grass genotypes assembled from two independent collections, a subset of 84 genotypes was used to develop a high-throughput in vitro screen for methane production scaled to allow the methanogenic capacity of forages to be screened at scale. Napier grass were selected and evaluated for 2 years in dry (DS) and wet (WS) seasons, and a minimum of 4 samples from each genotype on each season (leading to over 766 plant samples) were harvested, dried and chopped. Samples were assigned randomly to 4 different batches and 45 mg of each were incubated as feed with a 4.5 mL mixture of buffer solution and mixed rumen fluid from 4 cows fed a mixed forage diet. Incubation was performed in 20 mL vials at 39°C in anaerobic atmosphere. Orthophosphoric acid was added after 24h of incubation to stop fermentation, gas pressure was measured and samples ran through a GC system coupled with FID detector to measure methane.

Significant variation in methanogenic capacity of Napier grass accessions was observed with an almost 3 fold difference in methane yield from the highest to lowest methanogenic accessions (1833 v 5078 nMol methane per incubation over 24 hr which on initial analysis was not directly correlated to fibre digestibility suggesting that it will be possible to select for low methane accessions that promote animal production.

We observed clear differences in the methanogenic capacity of accessions caused by growth conditions with accessions grown in the wet season producing more methane than those grown in the dry season (4545 v 3060, SED 111, nMol methane per incubation over 24 hr). However, this is not true of all accessions and in some cases wet season-harvested plants produced less methane than the same accession grown in the dry season. This suggests that variation exists between accessions such that it should be possible to identify plant accessions that are resilient to climate effects in terms of both biomass production and lower methanogenic capacity. We will further investigate variation between accessions in methanogenic capacity to introduce new phenotypes for the improvement of forages. Future research will estimate the genetic correlation between traits and optimise methods to harness this novel source of variability for deployment in modern breeding and gene editing programs to drive a step change in the productivity and resilience of livestock production in SSA and Asia.

59 Methane emission from manure and blood metabolites of sheep fed basal diets and browse leaves

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Due to the relatively short life of methane in the atmosphere and its high global warming potential, reducing methane emissions is seen as a rapid way to help limit global warming to 1.5°C above preindustrial levels. Assessment of the concentrations of blood metabolites help to understand the nutritional and metabolic status of animals. Methane emission from manure and blood metabolites levels of sheep fed Super Napier grass (**SNG**), sodium hydroxide treated rice straw (**NaOHRS**), *Albizzia lebbek* (**AL**) and *Leucaena leucocephala* (**LL**) have not been extensively studied in Ghana. Therefore, the objective of this study was to determine methane emission from manure and blood profiles of sheep fed SNG, NaOHRS, AL and LL. The experiment was carried out at the Livestock and Poultry Research Centre, University of Ghana, Legon. Twenty-four forest type sheep were employed in the study, consisting of twelve ewe lambs and twelve ram lambs that were seven months old and had an average live weight of $10.56 \pm 1.2\text{kg}$. The animals were housed in separate pens that had cement floor, sufficient ventilation, and intensively managed for three months. The four diets fed were SNG (Control) [*Adlibitum*], SNG [*Adlibitum*] + NaOHRS [100 g], SNG [*Adlibitum*] + NaOHRS [100 g] + AL [200 g] and SNG [*Adlibitum*] + NaOHRS [100 g] + LL [200]. There was 14 days of feed, faecal bag and pen acclimatization before commencement of data collection. Manure (mix with urine in the case of ewes) was collected by fixing a faecal bag lined with polytene to the anal part of the sheep for 24 hours at the beginning, midway and end of experiment. Collected manure was stored by freezing until methane emission measurements. Methane emission from manure was measured using gas methane detector (GASTiger 2000, Stark Instrument Ltd, China). Blood samples (5ml) were taken from the jugular vein into gel and clot activator tubes at the beginning, midway and end of the experiment. Data obtained were analysed as completely randomised design and subjected to analysis of variance (**ANOVA**) using GenStat for windows version 12.1. Methane emission from manure was lowest ($p < 0.05$) in sheep fed SNG + NaOHRS + LL but differed ($p < 0.05$) from sheep fed SNG + NaOHRS + AL. Sheep fed SNG + NaOHRS had the highest ($p < 0.05$) methane emission from manure but was not different ($p > 0.05$) from sheep fed SNG. In terms of sex, manure from ewes emitted higher ($p < 0.05$) methane than the rams in all the treatments and this may be due to the collection of manure and urine together in the case of the ewes. Results showed that dietary treatment did not significantly ($p > 0.05$) influence most of the serum blood metabolites measured except alanine aminotransferase (U/L) and total bilirubin ($\mu\text{mol/L}$) which were different ($p < 0.05$). Blood metabolites evaluated were within the normal range for sheep. Therefore, the experimental diets did not have negative impact on the sheep. It is recommended that farmers should supplement their diets with browse leaves (AL and LL) because it has the potential to reduce methane emission from manure.

42 Effect of various multispecies pasture combinations on *in vitro* methane production for temperate dairy systems

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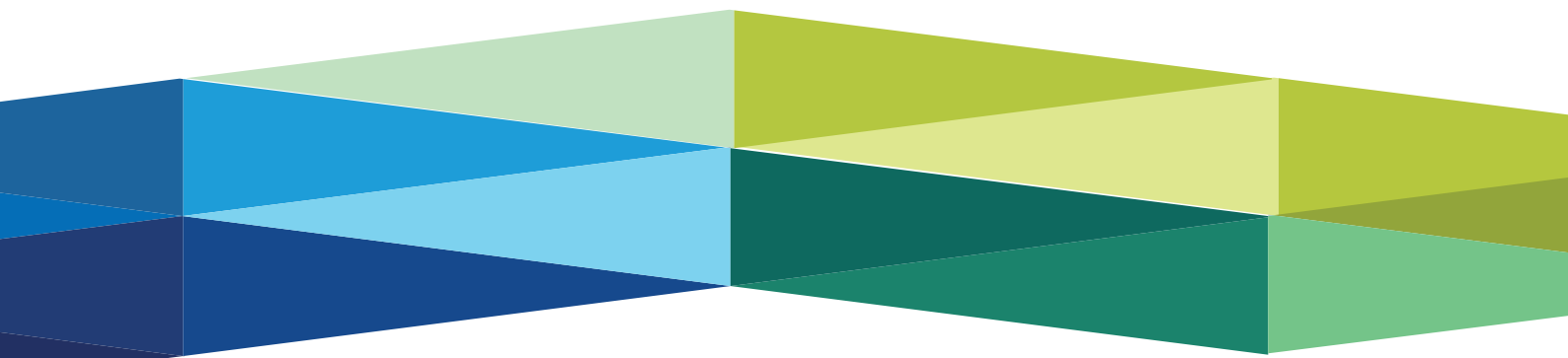
Grazing multispecies pastures can lead to reduced enteric methane emissions compared to grazing monocultures due to the presence of certain pasture species and their nutritional and plant secondary compound profiles. This study aimed to combine species of known individual methane mitigating potential that are suited to temperate dairy grazing in varying numbers and proportions to identify the complementarity or inhibitory effect of their plant secondary compounds on *in vitro* methane production. The study evaluated the effect of increasing species richness (3, 5 and 7 species) and varying functional group proportionality (ratios of grass: legume: herb) in a 3 × 4 factorial design and compared these with a control (perennial ryegrass). Functional group ratios tested were Even (33:33:33), Grass heavy (66:17:17), Legume heavy (17:66:17) and Herb heavy (17:17:66). The 3-species mixture consisted of perennial ryegrass, white clover, and burnet; the 5-species mixture additionally contained subterranean clover and yarrow; and the 7-species mixture added red clover and plantain to the above 5-species mixture. Samples were collected at a grazing ready stage for each respective species from the Ellinbank SmartFarm (Victoria, Australia) during Autumn 2024, oven dried at 60°C until constant weight, and ground to pass through a 0.5mm sieve. The *in vitro* study was conducted as a Randomised Complete Block Design in which four replicate incubation jars per treatment were blocked to one of four water baths split between two automated ANKOM^{RF} fermentation systems. The study was repeated three times giving 12 replicates per treatment. Each replicate consisted of 1g DM pasture in a 100ml buffered ruminal fluid inoculum (buffer: rumen fluid; 3:1) with cumulative total gas production recorded over 24h. Following incubation, headspace gas samples were collected, and methane concentration was analysed using gas chromatography. Data were analyzed using a general analysis of variance (**ANOVA**) with a nested treatment structure specified as *control/(species richness*functional group)*. This structure accounted for the main effect of the control, species richness, functional group proportion, and their interaction and the random effect of blocking structure was also fitted. Fisher's unprotected Least Significant Difference test was used for multiple comparisons. There was no significant interaction between functional group proportion and species richness on *in vitro* total gas and methane parameters ($P=0.6056$, $P=0.186$ respectively). However, there were significant effects of functional group proportionalities on *in vitro* total gas and methane parameters ($P < 0.001$). Herb Heavy was the only functional group that resulted in lower total gas and methane production respectively, compared to all other treatments tested (87.38ml/gDMI incubated (DMI) vs. 94.18 ml/gDMI on average; 9.68 vs. 11.31ml/gDMI on average; $P < 0.001$). Herb Heavy gave 8.6 % methane to total gas ratio reduction compared to control ($P < 0.001$). Incorporating herb dominant multispecies pastures into the temperate dairy feedbase is a promising approach for methane abatement compared to using perennial ryegrass monocultures, however, lower total gas production suggests digestibility of herb heavy may be lower, which requires further analysis. Furthermore, *in vitro* testing can only be used as preliminary screening, requiring confirmation through *in vivo* studies.



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1.5 Innovative feeding strategies for reducing methane emissions



172 Feeding a bromoform-based feed additive for methane mitigation in beef cattle

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Bromoform (**CHBr₃**) is a compound with strong methane-mitigating potential that binds to reduced vitamin B12, thereby inhibiting the cobalamin-dependent methyltransferase step of methane formation. Bromoform interacts with cofactor F430 of the methyl-coenzyme M reductase and inhibits the reductive release of methane (**CH₄**) from methyl-coenzyme M, another essential enzymatic reaction in the methanogenesis cycle. However, bromoform is highly volatile and requires stabilization. This experiment evaluated the CH₄ mitigating effect of feeding the Rumin8 Investigational Veterinary Product (**IVP**), a feed additive containing stabilized bromoform in an oil medium to beef cattle in a cross-over design experiment with two treatments and 10 animals, i.e., Control and Rumin8 IVP (with the active at 30 mg bromoform/kg DM). Rumen fluid and blood samples were collected 3 h post-feeding on the days steers exited the respiration chamber. On average, supplementing with Rumin8 IVP reduced CH₄ emission by 93% and CH₄ yield by 94% when compared to Control. There was a shift in the dissolved CH₄ and hydrogen (**H₂**) concentration in the rumen fluid samples consistent with the reduction in the CH₄ production and CH₄ yield. Supplementing Rumin8 IVP had no effect on feed dry matter intake or animal health parameters in the blood. The feed additive containing stabilized bromoform at 30 mg bromoform per kg DM appears to be a promising feed additive to reduce beef cattle's CH₄ emissions. However, further investigation is necessary to understand its long-term effect on the animal.

132 Novel calcium peroxide-based dietary additive improves beef cattle performance and reduces methane emissions

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Irish agriculture accounts for over 38% of national greenhouse gas emissions with the greatest contribution from enteric fermentation in ruminant livestock. To achieve a 25% reduction in agricultural emissions by 2030, the livestock sector requires feed management strategies that significantly reduce methane emissions from the national herd. A novel calcium peroxide-based dietary additive (**CaO₂**) can potentially reduce methane emissions in cattle. This study aims to evaluate the effect of dietary inclusion levels of a novel calcium peroxide-based feed additive on beef cattle's production performance and methane emissions. Seventy-two spring-born Charolais cattle (36 steers and 36 heifers) with an average body weight of 466kg and 15 months of age were randomly assigned to one of the four dietary inclusion levels of CaO₂, based on total dry matter intake. The control diet with 0% CaO₂ inclusion (**CON**), a low inclusion diet with 0.4% CaO₂ (**LID**), a medium inclusion diet with 1.2% CaO₂ (**MID**), and a high inclusion diet with 2.0% CaO₂ (**HID**) (n=18 per group). Animals received their respective treatments over a 12-week period, during which dry matter intake (**DMI**), average body weight (**ABW**), average daily gain (**ADG**), feed efficiency (**FCE**) and gaseous emissions (C-Lock Inc., Rapid City, SD) were measured. All animals were fed a basal diet containing 60% grass silage and 40% concentrate. The average weekly individual DMI, growth, and gaseous emissions data were analysed using linear mixed models in R with restricted maximum likelihood (**REML**). Fixed effects included treatment, week, and their interaction, while blocks were treated as random effects, with week as the repeated measure. The results were reported as least square means with significant differences at $P \leq 0.05$. Total DMI varies across the treatment groups with animals offered MID having the lowest (8.91 kg/day) compared to CON (9.10 kg/day) and HID (9.11 kg/day) ($P < 0.001$). Considering the growth performance metrics, ABW was highest for HID (507 kg) and lowest for MID (484 kg) ($P < 0.001$). Similarly, ADG increased with CaO₂ inclusion, reaching 1.16 kg/day in HID compared with 1.03 kg/day in CON ($P < 0.001$), resulting in the highest FCE for HID (0.13 kg ADG/kg DMI) ($P < 0.001$). Absolute CH₄ emissions were lower in MID and HID (211 and 209 g/day respectively) compared to CON (233 g/day) and LID (228 g/day) ($P < 0.001$). Animals offered HID had the lowest CH₄ yield with a reduction of 2.6, 2.3 and 0.6 g/kg DMI compared to the CON, LID, and MID treatment groups, respectively ($P < 0.001$). Similarly, the animals in the HID treatment group had lower CH₄ intensity up to 43, 52 and 13 g/kg of ADG when compared with CON, LID, and MID treatment groups, respectively ($P < 0.001$). Supplementing a forage-based diet with 2.0% CaO₂ improved growth rate and feed efficiency in beef cattle while reducing methane emissions and intensity up to 10% and 19%, respectively. This strategy offers a practical solution for the livestock industry to enhance production efficiency and reduce methane emissions.

95 Turnip leaf-bentonite nanocomposite as a novel feed additive to mitigate methane production

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Identifying natural alternatives to antibiotics for improving ruminant productivity and performance is essential for sustainable livestock management. This study develops and evaluates the physicochemical properties and biological effects of a turnip leaf (*Brassica rapa subsp*) phytochemicals -bentonite nanocomposite (**TLBN**) as a feed additive. The efficacy of TLBN in modulating ruminal fermentation and reducing methane emissions was compared to the ionophore monensin and normal bentonite. The wet impregnation technique was employed to incorporate the active components of turnip leaves into bentonite. The in vitro gas production (**GP**) assay was used to assess the biological effects of varying levels of the developed TLBN on ruminal fermentation, compared to monensin and natural bentonite. The experimental treatments included a control (no supplementation), monensin (40 mg/kg dry matter [**DM**]), natural bentonite (5 g/kg DM), and TLBN at 1, 2, and 3 g/kg DM, all added to the control basal substrate. All obtained results were statistically analyzed using one-way ANOVA with the SAS procedure. Gas chromatography–mass spectrometry (**GC-MS**) analysis identified 14 bioactive phytochemicals in the turnip leaf–bentonite nanocomposite, with benzoic acid being the most abundant (50.5%). Particle size distribution analysis showed a significant reduction in particle size, with D90 decreasing to 75 nm in TLBN. The TLBN exhibited a larger specific surface area, higher electrical conductivity, and distinct morphology, as observed through electron microscopy, compared to natural bentonite. The results of the in vitro experiment indicated that TLBN supplementation at 3 g/kg DM resulted in the lowest ($P < 0.05$) total GP values, with a reduction in methane production comparable to monensin when compared to the control diet. No significant differences were observed among the experimental diets in terms of nutrient degradability or ammonia concentration. All experimental feed additives significantly reduced ($P < 0.05$) total protozoal count compared to the control. Monensin reduced ($P < 0.05$) total short-chain fatty acids (**SCFAs**) and acetate levels, while both monensin and TLBN at 3 g/kg DM enhanced propionate production. These results highlight the potential of TLBN as a novel feed additive capable of replacing monensin in ruminant diets to reduce methane emission.

152 Efficacy of 3-NOP applied in drinking water on enteric methane reduction in sheep

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The dietary most effective strategy to reduce methane (**CH₄**) emissions is incorporating additive CH₄ inhibitors into ruminant diets. However, there is a need to broaden the actual presentation form (i.e., solid powder) of the commercially available products to allow their use in grazing systems. This study aimed to test the efficacy of 3-NOP on enteric CH₄ reduction in sheep when applied in drinking water as a possible delivery system in livestock grazing conditions. The study followed a 4 x 4 Latin square design, enrolling 4 non-lactating female adult Segureña sheep with an average body weight (**BW**) of 44.9 ± 2.6 kg. The study lasted 8 weeks, with 4 experimental periods of 2 weeks each. The experimental periods consisted of 7 days of adaptation to the treatments and 3 days of enteric gas emission measurements (CH₄ and H₂) in open-circuit respiration chambers, followed by a wash-out period of 4 days. Sheep were randomly assigned to 1 of the 4 experimental treatments. The treatments were: i) a basal diet, 70% perennial ryegrass hay and 30% concentrate, without supplementation (**CTL**); ii) a basal diet supplemented with 80 mg of 3-NOP per kg of dry matter intake (**DMI**) (Bovaer® 10) as recommended dose by the manufacturer; iii) a basal diet supplemented with 80 mg of 3-NOP in the drinking water (**DW80**) per kg of DMI; and iv) a basal diet supplemented with 120 mg of 3-NOP in the drinking water (**DW120**) per kg of DMI. There were no significant differences ($P > 0.05$) either in the feed or water intakes across treatments. Methane emissions (g/d) and yield (g/kg DMI) were significantly lower in Bovaer® 10 (-26.8%), DW80 (-41.4%), and DW120 (-35.7%) than in CTL ($P < 0.05$). Sheep in Bovaer® 10 treatment had a greater impact on reducing emissions in the first 2 hours after feeding times and a smaller reduction in the following 2 hours. In contrast to a pulse dose of Bovaer® 10, the impact of treatments in DW80 and DW120 lasted for 4 hours post-feeding. For the second half of the day (i.e., 4 hours after the second feeding), Bovaer® 10, DW80, and DW120 had lower emission values than CTL. Sheep in DW80 had lower emission values than Bovaer® 10 in concordance with their drinking pattern and, thereby, the treatment intake. Results suggest that adding 3-NOP to the drinking water could be a potential strategy to supply it in grazing systems where feed formulation does not necessarily allow to deliver 3-NOP mixed with the diet.

293 Effect of feed additives on methane emissions of finishing steers under grain-based diet

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The interplay between anthropogenic greenhouse gas (**GHG**) emissions and global warming is critical, particularly in regions like Uruguay where livestock farming significantly contributes to emissions. Addressing enteric methane from beef cattle is essential not only for mitigating climate change but also for promoting sustainable beef production and economic development in agricultural sectors. This study assessed the impact of various feed additive supplements on enteric methane emissions and production performance of beef cattle in a commercial feedlot under a grain-based finishing diet consisting of a total mixed ration (**TMR**) of steam-flaked and whole corn grain, soybean extrudate, sawdust, bark, and urea. Twenty-eight Angus steers (body weight of 512 ± 22 kg) were blocked and randomly assigned to four additive treatments ($n = 7$ animals/treatment): 1. Control (Monensin, 1.65 g/kg dry matter - DM); 2. 3-NOP (10.35 g/kg DM); 3. mixture of tannins and essential oils (1.575 g/kg DM); and 4. tannin extract (1.9 g/kg DM). Animals were kept in individual pens and fed with the TMR two times daily for 93 days. Methane emissions were measured on each animal using the SF₆ tracer gas technique over two periods of 5 consecutive days each, which started 36 and 71 days after the administration of additives, respectively. Dry matter intake (**DMI**) and body weight (**BW**) were registered daily and every 14 days, respectively. A mixed linear model was used to analyze methane emissions and performance variables, with mean comparisons via the Tukey-Kramer Test (significance was established at $P < 0.05$). The results indicated that the feed additive 3-NOP significantly reduced both absolute methane emissions, **Y_m**, and emission intensity ($P < 0.05$). In contrast, the other additives did not show a significant impact on methane emissions. Animal performance variables such as DMI, final BW, and average daily gain (**ADG**) were not significantly affected by the administration of any of the additives. When compared to the control group, the administration of 3-NOP resulted in a reduction of absolute methane emissions per day by 32.5%, and methane yield (g/kg DMI) by 34.0% ($P < 0.05$). In general, 3-NOP shows a promising result for reducing methane emissions in steers under a grain-based finishing diet, without compromising their growth performance. Further research is needed to explore the dose and long-term effects of 3-NOP on methane emissions and production performance of feedlot cattle at a commercial scale.

268 Preliminary investigation: Role of inoculum characteristics on *in vitro* effectiveness of 3-nitrooxypropanol on methane mitigation

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Mitigating enteric methane (**CH₄**) from ruminant production is a key strategy for achieving short-term climate change alleviation goals. Among anti-methanogenic compounds, 3-nitrooxypropanol (**3-NOP**) has emerged as a promising feed additive. The effectiveness of 3-NOP is inherently dose-dependent; however, it may also be influenced by the basal diet, animal species, and microbial community. This study employed a meta-analytical approach to elucidate the variability in absolute and relative *in vitro* CH₄ production responses to 3-NOP dosage and inocula characteristics. Data from nine *in vitro* experiments, each using mixed rumen inocula from 3 dairy cows as donor fluid, met the inclusion criteria. Donor cows received a basal diet (maize silage and wilted grass silage, 50% each, dry matter (**DM**) basis) plus supplements to meet the animals' requirements for milk production. Predictors were mean-centred, including 3-NOP dose and inocula characteristics (pH and acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproate proportions (mol/100mol)). Average, minimum and maximum values of the selected predictors were: 0.89, 0.60 and 1.20 (mg/g DM substrate) for 3-NOP dose; 6.38, 6.07 and 6.88 for inocula pH; 67.5, 63.5 and 73.2% for acetate proportion; 13.2, 10.4 and 15.3% for butyrate proportion; 1.23, 1.02 and 1.64% for isovalerate proportion; and 0.57, 0.47 and 0.78% for caproate proportion. Inhibition was calculated as the control mean minus 3-NOP treatment, expressed as a percentage of the control mean. Model fitting was developed in 2 categories: 1) 3-NOP dose and individual covariates and 2) selected models from all covariate combinations. Models with covariates showing $P < 0.1$ and absolute correlation with other covariates of less than 0.5 were evaluated using the leave-one-out cross-validation (**LOOCV**) method. At the average dose of 3-NOP at 0.89 mg/g DM substrate, reductions ($P < 0.001$) of 52.7 and 53.7% were observed for absolute CH₄ production ($\mu\text{mol/g DM substrate}$) and relative CH₄ production (CH₄/VFA, mol/mol), respectively. Individually, at the mean 3-NOP dose, all inocula characteristics affected both absolute and relative CH₄ production. In the selected model, each 1%-unit increase in acetate and 0.1%-unit increase in caproate proportion from their means enhanced by 7.45% and 4.86% reduction in absolute CH₄ production, respectively, at a given dose of 3-NOP. For absolute CH₄ production, the LOOCV RMSE decreased from 19.3% (model with 3-NOP dose only) to 9.45% (model including 3-NOP dose, acetate, and caproate proportions), while the Corrected Akaike Information Criterion (**AICc**) reduced from 260 to 232. For relative CH₄ production, a 1 pH-unit increase in inocula pH from its mean improved 3-NOP inhibition efficacy by 4.00%, increases of 1%-unit in butyrate proportion and 0.1%-unit in isovalerate proportion from their means decreased 3-NOP effectiveness by 13.3% and 3.78%, respectively. The LOOCV RMSE reduced from 18.5% (model with 3-NOP dose only) to 8.60% (model containing 3-NOP dose, inocula pH, butyrate, and isovalerate proportions), while AICc dropped from 246 to 220. These findings highlight that 3-NOP efficacy is simultaneously influenced by inocula characteristics rather than being driven solely by dosage. Further analysis will explore other interactions, such as the incubated substrate, to refine predictive models.

279 Use of calcium peroxide to inhibit methane from Norwegian White Sheep

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In 2019, the Norwegian Farmers' Association and the government agreed to reduce greenhouse gas emissions in agricultural sector by 5 million tonnes of CO₂ equivalents by 2030. The Agriculture Climate Plan (2021-2030) states that half of this reduction will be achieved through feeding and breeding measures, including methane inhibitors in ruminant feed. According to the Agricultural Agreement between Agriculture and the State for 2025, all ruminants in Norway will be provided with methane inhibitors by 2027. Testing in Ireland and Northern Ireland has shown that calcium peroxide (CaO₂) can reduce methane emissions by 28% in sheep, cows, and steers, without affecting feed intake or productivity. Calcium peroxide is hydrolysed to calcium hydroxide and hydrogen peroxide, which is further degraded to oxygen and water. Oxygen in the rumen creates an unfavourable environment for anaerobic organisms (including methanogenic bacteria) to form methane. In Norway, CaO₂ was used in a dose-response trial with 30 Norwegian white sheep. The ewes were stratified by live weight and randomly assigned to one of three diets: a control diet consisting of grass silage and crushed BeetBarley (concentrate), and two experimental diets that included CaO₂ at 1% and 2% inclusion on a dry matter basis, delivered through the concentrate feed. The amount of CaO₂ was adjusted twice weekly, based on individual feed intake. In a staggered order, six ewes (two from each diet) were introduced and adapted to their respective diets for 19 days, followed by 72 hours of methane measurements (CH₄: g/day) in open-circuit respiration chambers. Silage and concentrate intakes were recorded individually and converted to a dry matter basis. Variables analysed included total dry matter intake (**TotalDMI**: kg/day), the ratio of concentrate to silage consumption (**CON:SIL**), and g CH₄/ kg TotalDMI. A mixed model analysis was conducted to evaluate the effects of CaO₂ as a methane inhibitor across the three diets. Results indicated no difference in TotalDMI among the three groups (2.63 kg/day; p=0.93). However, the ratio of CON:SIL revealed significant differences, where the 2% inclusion group consumed less concentrate than the Control and 1% inclusion group (0.15, 0.15, and 0.13, for Control, 1%, and 2% CaO₂ inclusion, respectively; p=0.03). Moreover, CH₄ emissions were significantly different among the diets, with the Control group emitting the highest amount, and significantly more than the experimental diets that did not differ from each other (24.55, 21.80, and 20.64 g/day, for Control, 1%, and 2% CaO₂ inclusion, respectively; p<0.01). A trend was observed with g CH₄/kg TotalDMI, across the diets (9.80, 9.05, and 8.40 g/kg, for Control, 1% inclusion, and 2% inclusion, respectively; p=0.08). However, the analysis revealed a significant difference between the Control diet and the 2% inclusion (p=0.02), with a notable reduction of 14.30% observed for the 2% inclusion compared to the Control. In conclusion, the 2% inclusion of CaO₂ shows promise as an inhibitor of CH₄ emissions in sheep fed silage. However, it is important to note that the 2% inclusion group consumed significantly less concentrate during the 72-hour.

251 Identification of anti-methanogenic compounds through in silico docking and in vitro ruminal fermentation

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Methane (**CH₄**) is produced in ruminal fermentation as part of hydrogen elimination via CO₂ reduction in methanogenic archaea. The final step of methanogenesis is catalysed by Methyl-coenzyme M reductase (**MCR**), which reduces methyl-CoM with coenzyme B to release CH₄. Structure-based virtual screening is a widely used approach in drug discovery to identify potential enzyme inhibitors by computationally predicting their binding affinity. Such techniques, applied to mitigate CH₄, have the potential to preliminarily identify specific inhibitors of methanogenesis. Although numerous targets exist for potential CH₄ inhibition, targeting MCR seems the best compromise, minimizing interference with other pathways operated by ruminal microorganisms while not requiring overly complex or large inhibitory ligands. Currently, limited compounds were discovered and validated targeting methanogenesis (e.g., 3-Nitrooxypropanol), and to our knowledge, no natural products have been commercialized with such a declared effect.

The objective of this study is to develop and validate a computational approach using well-developed docking algorithms and compound libraries to identify potential MCR inhibitors, while also creating an open-source database to support prediction and validation of CH₄ inhibitors. Such a platform will provide rich open data to the community for the prediction and validation of potential CH₄ inhibitors. Leveraging an in-silico approach, our in-silico screening involved over 180,000 compounds sourced from plant, food, milk, yeast, and bovine-related databases (FooDB, YMDB, BMDb, HMDB, MCDB). Compounds were pre-filtered to exclude those with undesirable properties such as Pan-Assay Interference Compounds (**PAINS**) and unfavorable ADMET profiles. The remaining compounds were clustered based on structural similarity to streamline the dataset. Using Autodock Vina, we predicted potential MCR binders and performed molecular docking simulations with the high-resolution crystal structure of MCR (1.10 Å, PDB ID: 5A0Y), focusing on a predefined binding pocket where methyl-CoM and CoB interact. To improve selectivity and reduce off-target effects, we predicted binding for seven enzymes involved in glycolysis and short-chain fatty acid pathways, eliminating compounds with potential side effects. From this process, we identified 896 potential MCR inhibitors.

According to predicted efficacy, inhibitory potential, and cost-effectiveness of the identified candidate inhibitors, we plan to select up to 24 compounds to conduct a 24-h in vitro ruminal fermentation with Hohenheim gas test. This validation experiment is ongoing. Rumen fluids will be collected from rumen-fistulated dairy cows before morning feeding and a common lactating dairy cow diet is used. Gas production concentrations, in vitro organic matter degradability, and the fermentative profile will be assessed for a complete evaluation of the predicted anti-methanogenic potential of the molecules. Findings will be presented at GGAA Conference 2025, and the complete database will be made open source in the following publications. Further studies are needed to assess if the developed computational approach can be used to target additional active sites involved in ruminal methanogenesis.

180 Enteric methane and milk production – effects of fat and forage sources

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According to Danish legislation, farmers must feed lactating non-organic dairy cows either at least 48 g fatty acids (**FA**)/kg DM all year or 60 mg 3-NOP/kg DM during 80 days per year to mitigate enteric methane. The aim was to study the impact of supplementing rapeseed or saturated fat to diets with different grass-clover:maize silage ratios on enteric methane and milk production.

It was a continuous experiment with a 3-week covariate period and a 4-week treatment period. Emission of enteric methane was measured by GreenFeeders. All diets comprised 60% DM from silage, with 2 silage combinations: either **GRASS**, 80:20 grass-clover:maize silage in % of silage DM or **MAIZE**, with the ratio 20:80. Within each silage combination 3 fat treatments were applied in a factorial design, **CON** with no added fat (22 g FA/kg DM in the diet) or **SAT** with addition of 8 g FA/kg DM from rapeseed cake and 16 g saturated FA (45:45:10 palmitic:stearic:oleic acid) or **RAP** with addition of 16 g FA/kg DM from rapeseed cake and 8 g saturated FA comprising 46 g FA/kg DM. Starch content was 212 and 150 g/kg DM in MAIZE and GRASS diets, respectively, whereas NDF content was 330 g/kg DM in all diets. Ninety-six cows were blocked according to parity (1st or 2nd) and days in milk. Data from the last 2 weeks were used for statistical analyses, which were performed by R version 4.0.4 with a model including the fixed effects: covariate for the response variable, silage, fat, and interaction between silage and fat and block as a random effect. Differences were considered significant if $P < 0.05$.

There was no interaction between silage and fat combinations for DMI and methane yield (per kg DMI). Due to wet grass-clover silage, DMI was significantly lower for the 3 GRASS diets with an average intake of 21.1 kg DM compared to 24.2 kg for the MAIZE diets. There was a significant effect of fat on DMI (22.7, 22.3 and 23.1 for CON, SAT and RAP, respectively). The average methane yield was significantly higher (18%) for GRASS than for MAIZE diets. The effect of fat on methane yield was not significant, hence the addition of SAT and RAP only reduced methane yield by 1 and 3%, respectively compared to CON. There was an interaction between silage and fat with respect to milk yield and energy corrected milk (**ECM**), since GRASS diets with CON and SAT led to lower yields than all three MAIZE diets, whereas the GRASS diet with RAP did not. The highest ECM yield was found for MAIZESAT and MAIZERAP (both 38.9 kg), whereas the significantly lowest were found for GRASSCON (34.4 kg) and GRASSSAT (33.9 kg). Milk fat% was significantly higher for GRASS than for MAIZE diets, and higher for SAT than for CON and RAP diets.

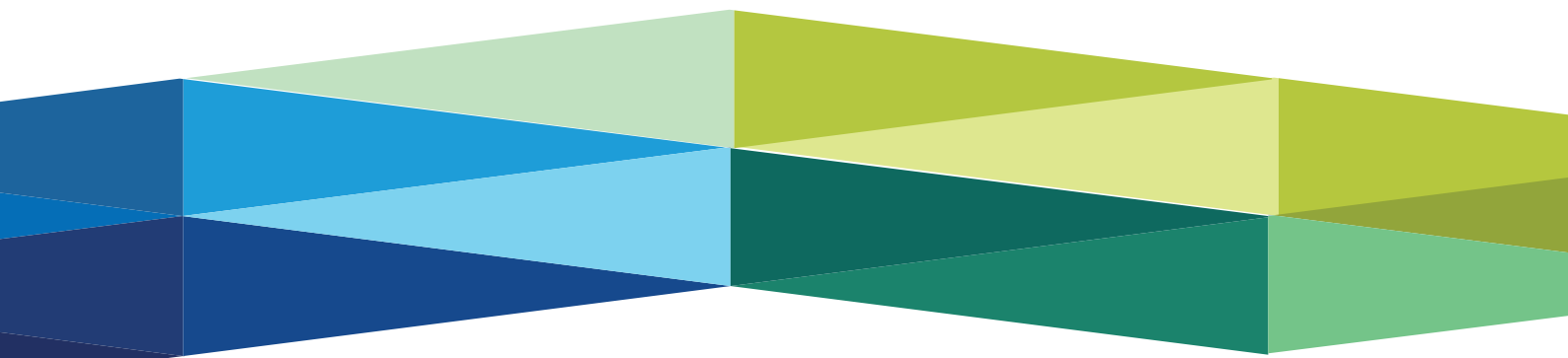
In conclusion, contrary to earlier studies, adding fat did not significantly reduce methane yield. The effect of fat on ECM interacted with silage type.



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1.6 Nutritional approaches to minimize greenhouse gas emissions



66 Animal Performance and Methane Emissions in Feedlot vs. Traditional Pastoral Systems with Concentrate Supplementation for Tanzanian Short Horn Zebu and Boran Cattle

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This study investigated the impact of concentrate supplementation in traditional pastoral systems on beef cattle performance and methane (**CH₄**) emissions in Tanzania. The study utilized reconstructed data based on a summary of an experimental study conducted in Tanzania. The experiment involved 60 Boran and 60 Tanzanian Shorthorn Zebu (**TSHZ**) cattle, which were assigned to five dietary treatments for 100 days: grazing alone (GrazC00), grazing with 50% concentrate (GrazC50), and ad libitum hay with 60%, 80%, and 100% concentrate supplementation (HayC60, HayC80, and HayC100, respectively). Dry matter intake (**DMI**) and live weights were measured throughout the experimental periods, and the carcass gain (**CaG**) and dressing percentage (**DP**) were assessed during slaughter. Daily methane production (**DMP**) was estimated from the reconstructed data, following the Intergovernmental Panel on Climate Change guidelines. The estimated CH₄ emissions were used to estimate CH₄ yield (g CH₄/kg DMI) and emission intensities (**EI**) about weight gain and CaG (**EIWG** and **EICaG**, respectively). Statistical comparisons were conducted using t-test and ANOVA, with significance at $p < 0.05$. Results showed that Boran cattle had a higher range of average daily gain (**ADG**) and DMP compared to TSHZ, with concentrate supplementation leading to increases of 5.6% to 27.0% in ADG and 2.0% to 13.0% in DMP. Boran cattle also exhibited a higher range of DMI and CaG compared to TSHZ, with increases of 19% to 29.5% in DMI and 10.3% to 35.1% in CaG. Boran exhibited higher range of DMP by 28%, 21%, 12%, 35 % and 13 % compared to TSHZ for GrazC00, GrazC50, HayC60, HayC80, and HayC100 treatments, respectively. In both breeds, EIs decreased in the range of 20% to 55% by increasing concentrate levels from 0% to 100 %, with the lowest EIWG observed in the HayC100 treatment (401 to 90 g CH₄/kg WG), and the lowest EICaG also observed in the HayC100 treatment (808 to 161 g CH₄/kg CaG). However, no differences in the range of EIs were observed between the two breeds across the treatments. Overall, concentrate supplementation improved cattle performance and reduced DMP and EIs. These findings suggest that concentrate supplementation could be an effective strategy for enhancing beef production efficiency and reducing environmental impact in Tanzania. However, economic evaluation is needed to determine optimal supplementation levels for practical application.

126 Genetically modified high-lipid ryegrass silage reduced methane emissions in sheep

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Genetically modified High Metabolisable Energy Ryegrass (**HME**) was developed with a high lipid content to increase the metabolizable energy content of pasture. Feeding other sources of lipids was previously found to reduce enteric methane emissions from ruminants. The objective of the current study was to determine methane emissions from sheep-fed HME silage compared to a control ryegrass silage (**NULL**). HME could only be grown in a PC2 glasshouse and therefore HME and NULL were harvested multiple times over 18 months and preserved as silage to produce sufficient feed (~100 kg DM each) for a small study with 24 sheep. Sheep were initially adapted to commercial ryegrass silage and a background methane measurement was performed before transitioning 24 sheep to HME and NULL silage for two days followed by nine additional days on treatment silages before methane measurements. The feed offer was restricted to 0.8 kg DM/day because of limited feed available and to minimize between sheep variation in dry matter intake (**DMI**). Gas emission and DMI measurements were performed for 48 h in 24 respiration chambers organised in three systems with eight chambers connected to a gas analyser. A rumen sample was collected via oral stomach tubing after chamber measurements (before morning feeding) and volatile fatty acids were analysed. Data were analysed using a linear mixed model (in R Core Team, 2023) with silage treatment as a fixed effect, chamber system as a random effect and the background measurement for each variable as a covariate. The crude fat content was 2.2% units greater and gross energy was 0.7 MJ/kg DM greater in HME silage than in NULL silage. The methane as a percentage of gross energy intake was 11% less in sheep-fed HME silage compared to those fed NULL silage ($P = 0.026$) and tended to be 9% less when expressed as g/kg organic matter intake ($P = 0.091$). Gross CH₄ emissions (g/d) and CH₄ as g/kg DMI were numerically 9% less ($P = 0.133$) and 7% less ($P = 0.201$), respectively, in sheep-fed HME silage compared to those fed NULL silage. Acetate proportion was 5% less ($P = 0.019$) and propionate proportion 20% greater ($P = 0.005$) in sheep-fed HME compared to sheep-fed NULL silage. The results of the current study indicate that feeding high-lipid ryegrass to sheep has the potential to reduce methane emissions compared to those fed conventional ryegrass.

356 Early life interventions in dairy calves for persistent methane mitigation

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Priming the rumen microbiome of calves during its early development towards a lower methane emission may result in persistent effects on emissions later in life. Such priming could be obtained using anti-methanogenic feed additives. Bovaer, including the active component 3-nitrooxypropanol (**3-NOP**), has a known anti-methanogenic mode of action with well-documented effects on methane emissions in cattle. Dimethylglyoxime (**DMG**), a nickel-selective chelating agent has been shown to reduce the bioavailability of nickel, an element which is essential for methanogenesis, but which has no known nutritional requirement in cattle. This is the first study to investigate the in vivo effects of DMG on methane emissions in cattle. The objective of this study was to quantify the effects of Bovaer and DMG in calves and to investigate if such early life interventions would have persistent effects on methane emissions later in life. Thirty-two Danish Holstein heifer calves were enrolled in a continuous block design from day 1 of life. Calves were blocked according to birth order and randomly allocated to 1 of 4 dietary treatments. The treatments were 2×2 factorially arranged with 2 levels of Bovaer (0 or 225 mg 3-NOP/kg dry matter) and 2 levels of DMG (0 or 6,500 mg/kg dry matter). The treatments were included in a total mixed ration with a forage to concentrate ratio of 15:85 on a dry matter basis. Diets were available ad libitum throughout the first 12 weeks of life. During week 1 and 2, when solid feed intake was low, treatments were administered daily in a liquid suspension (10.5 mL) dosed orally 2 hours after morning milk feeding. Calves were offered milk replacer in equal portions 2 times daily (up to 8 L/day) and were gradually weaned from week 6 to week 8 of life, where calves were fully weaned. Calves were housed individually in pens bedded with straw. Milk replacer intake was registered daily, whereas solid feed intake was measured 2 times per week. Calves were weighed weekly. At 5 and 10 weeks of life, ruminal fluid, blood, and faeces were sampled. At 10 weeks, methane emission was measured using 4 respiration chambers. Calves were placed in each chamber in treatment-pairs for 4 consecutive days. This trial is ongoing, but preliminary data indicate that feed intake and body weight gain were not negatively affected by the treatments. Methane measurements suggest clear inhibitory effects of Bovaer supplementation during the treatment period, whereas the effects of DMG are less clear. A post-treatment follow-up methane measurement is undertaken when calves are 17-20 weeks old. These results will document if persistent methane mitigation can be achieved by early life interventions affecting the rumen microbiome.

62 Effects of cottonseeds and silage composition on methane emissions and milk production of dairy cows

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Supplementing cottonseeds and increasing the maize silage to grass silage ratio can decrease enteric methane emissions in dairy cows. This study evaluated the interaction between these strategies to enhance their methane mitigating potential. The trial was conducted using 8 Holstein Friesian cows in a replicated 4 × 4 Latin square design with four 28-day periods. Cows were randomly assigned to one of four treatments arranged in a 2 × 2 factorial design. Silage ratio treatment were 30:70 or 70:30 maize silage (**MS**) to grass silage (**GS**) ratio (**DM** basis). Cottonseed treatments were a control concentrate without cottonseed (**CON**), or one with 10.6 % dietary DM whole cottonseed with lint (**CTS**). All four TMR diets (68:32 silage to concentrate, DM basis) were offered *ad libitum*. During the last 7 d of each period, cows were housed individually, to measure feed offered and refused, and milk production and composition. Enteric methane was measured using the sulphur hexafluoride technique, and total faeces and urine were collected to assess nutrient utilisation. Results were analysed based on the fixed effects of the treatments and their interaction, and the random effect of period and cow.

The MS diets contained 13.8% less NDF, 14.2% more CP, and 34.7% more starch than GS. The CTS diets had 106% more EE and 12.3 % less starch than CON. Dry matter intake were higher with MS than GS (+0.70 kg DM/d; $P = 0.037$). Cottonseed diets reduced DM intake compared to CON (-0.89 kg DM/d; $P = 0.011$) but EE intake remained higher with CTS ($P < 0.001$). Total methane emissions decreased by 18% with CTS when MS was fed (459 vs. 378 g/d for MS-CON and MS-CTS, respectively), but not when GS was fed (446 vs. 427 g/d for GS-CON and GS-CTS, respectively) (silage × cottonseed interaction, $P = 0.06$). Similar trends were observed for CH₄ yield (g CH₄/DMI; interaction, $P = 0.065$) and Y_m (MJ CH₄/100 MJ GE intake; interactions, $P = 0.072$). Methane intensity decreased by 15.8% with MS (24.9 vs. 21.0 g CH₄/kg milk yield; $P < 0.001$) but was unaffected by CTS (23.1 vs. 22.8 g CH₄/kg milk yield; $P = 0.67$). The MS diets increased milk yield (+2.4 kg/d, $P < 0.001$) and milk protein content (+0.16 %; $P < 0.001$). In contrast, CTS reduced milk yield (19.7 vs. 18.0 kg/d; $P \leq 0.01$) but increased milk fat content (+0.24%; $P = 0.02$). The MS diets increased N excretion in urine by 31.4% ($P = 0.048$). Efficiency of N utilization (milk N/N intake) decreased with CTS ($P \leq 0.01$).

In conclusion, cottonseed decreased absolute enteric methane emission and yield in dairy cows when maize silage was the predominant forage but had no effect with grass silage. However, cottonseed also decreased milk production. In contrast, maize silage lowered methane intensity and improved milk production performance.

353 Developing methane conversion factor (Y_m) for Kenyan dairy cows: diet quality's on emissions and productivity

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Methane (CH_4) is the primary greenhouse gas (GHG) emitted by livestock in the agricultural sector, and accounts for 32% of all global anthropogenic GHG emissions. As part of their commitment to the Paris Agreement and the United Nations Framework Convention on Climate Change, Kenya has pledged to reduce 32% of its national GHG emissions by 2030 relative to the business-as-usual (BAU) scenario, where enteric CH_4 is the largest source of emissions, with dairy cows being a significant contributor. To estimate enteric methane emissions for national GHG inventories, the daily gross energy intake (GEI) of individual animals is calculated and then multiplied by a CH_4 conversion factor (Y_m). According to the Intergovernmental Panel on Climate Change (IPCC) Tier 2 methodology, key factors to estimate livestock GHG emissions include ruminant diets containing 18.45 MJ of gross energy (GE) per kg of dry matter (DM) and methane energy content of 55.65 MJ kg^{-1} . A standard Y_m value of 6.5-7.0% corresponding to 19.8 g CH_4 kg^{-1} DM intake has been adopted for Kenya's latest inventory of GHG emissions from dairy cattle. These default values are based on data collected from the Global North representing the livestock systems found there, but they have been implemented by many Sub-Saharan countries in their national GHG inventories, although the conditions in local systems might be different. This highlights an urgent need for local data to improve the accuracy of national inventories for SSA countries and to support governments in making informed decisions to meet mitigation targets. This study measured Y_m (CH_4 kg^{-1} DMI) of lactating cows fed diets based on local feed resources under semi-intensive conditions. Nine crossbred heifers were included in a complete changeover design (3×3 Latin square) with three experimental feeding periods of 20 days each (14 days adaptation followed by six days of sample collection). Samples were collected over three days for DM intake (DMI) and diet digestibility, and an additional three-day period in a respiratory chamber to measure enteric CH_4 emissions. Three experimental diets (0.88:0.12 forage-to-concentrate ratio on a DM basis) varying in crude protein (CP; % DM basis) and digestible energy contents were formulated as: 'Low' - (CP: 10%, DE: 59.5%), 'Medium' - (CP: 12%, DE: 61%), and 'High' - quality (CP: 14%, DE: 62.5%) diets. The Medium-quality diet served as the baseline for diets' formulation as reported in Kenya's GHG inventory and diets were aimed to be representative of local production conditions. Feed ingredients consisted of native pasture hays (low, medium, and high qualities), constituting approximately 50% of the diet, Napier grass silage as cultivated fodder (low, medium, and high qualities), crop residues (maize stover, banana leaves, sugarcane tops), weeds/protein-rich fodders (sweet potato vines, wandering Jew, lucerne), purchased feeds (maize germ, soybean meal), and a mineral mix. The trial is ongoing and will last until February 2025. The findings will be presented at the conference. This study will contribute to the understanding of how diet compositions in semi-intensive dairy systems can influence enteric CH_4 emissions to obtain a more reliable Y_m factor under Kenyan conditions.

154 Annual forage crop blends for grazing: impacts on forage yield, animal performance and methane emissions

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Simultaneous cultivation of multiple annual forage crops together (polycropping) for grazing with the interest of improving forage productivity and quality, soil fertility, animal performance and greenhouse gas mitigation are of increasing interest to Canadian beef producers. The objective of this study was to evaluate the impacts of using mixtures of annual forage crops for summer grazing on forage biomass yield and quality, animal performance as well as enteric methane emissions. The study was conducted at the Agriculture and Agri-Food Canada, Swift Current Research and Development Center, SK, Canada. Evaluated forage systems (treatments) were: 1) simple blend with two species mix (simple): CDC Haymaker oats (*Avena sativa*) + CDC Jasper pea (*Pisum sativum*), and 2) complex blend with eight species mix (complex): CDC Haymaker oat + CDC Maverick barley (*Hordeum vulgare*) + CDC Jasper pea + Hairy vetch (*Vicia villosa*) + Driller radish (*Raphanus sativus*) + Winfred forage brassica (*Brassica oleracea* × *Brassica rapa*) + Golden German millet (*Setaria italica*) + Phacelia (*Phacelia tanacetifolia*). After proper field preparation, treatments were seeded on June 7 and 8, 2023 on a 10.3 ha field subdivided into six, 1.72 ha grazing paddocks. Treatments were established in a randomized block design ($n = 3$). Grazing was conducted over 6 weeks period (24 July to 31 August, 2023) by randomly allocating 12 yearling beef steers per treatment (average initial BW = 363 ± 8 kg). The first two weeks of the grazing period were used as a covariate. Before the start of grazing, a total of 15 quadrat samples per paddock were clipped to determine DM yield, species composition and forage quality. Samples of residual were also collected at the end of grazing to estimate intake and utilization rate. Animal body weight was measured at the beginning and end of the grazing period as well as every two weeks during grazing. Enteric methane emission was measured using the sulfur hexafluoride (SF_6) technique in week three and five (2 periods) over four days. Data were analyzed using SAS (9.4). Forage DM yield was not affected by treatment ($P \geq 0.23$), 2.1 t/ha for simple vs 1.6 t/ha for complex. Furthermore, forage neutral and acid detergent fibre contents were similar between treatments. The contribution of cereals (*i.e.*, oats, barley, millet) in the forage mix was similar between treatments (73.8% for simple vs 78.9 for complex, $P = 0.15$) while the simple treatment contained more legume (peas, 15.3%) in the mix as compared to complex (2.5%, $P < 0.001$). Similarly, grazing animal performance was not affected by treatment ($P = 0.87$), 108 kg/ha for simple vs 106 kg/ha for complex treatment. Furthermore, methane yield (544 vs 664 g/kg average daily gain) was not affected by treatment ($P = 0.14$) while enteric methane emission (g/day) was higher ($P = 0.003$) for complex (315) treatment relative to simple (272). The preliminary results of our study suggested that either simple or complex annual crop mixtures can be used as forage alternatives for summer grazing without affecting forage biomass yield and nutritional quality, animal performance and enteric methane emission.

221 How can the use of tree legume forages be optimized in enteric methane mitigation strategies?

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Ruminant livestock contribute to global warming through enteric methane (**eCH₄**) emissions. Tree legumes, such as *Leucaena leucocephala* and *Gliricidia sepium*, have been reported to mitigate greenhouse gas emissions. This study aims to assess their potential for reducing eCH₄ emissions in cattle *in vivo*. To this end, ten Sudanese Fulani zebu cattle (5 cows of 206.5 ± 25.47 kg and 5 steers of 120.1 ± 19.17 kg) were used at **CIRDES** (International Centre for Research-Development on Livestock in the Subhumid Zone) in Burkina Faso, each receiving daily diet equal to 3.5% of its body weight (**BW**) on a dry matter (**DM**). The trial was conducted from November 2024 to April 2025 in 5 phases of 26 days each (14 days of diet adaptation and 12 days of data collection). Five diets were successively tested: R1 (85% *P. maximum*, 15% *Leucaena leucocephala*), R2 (85% *P. maximum*, 15% *Gliricidia sepium*), control R0 (100% *P. maximum*), R3 (70% *P. maximum*, 30% *Leucaena leucocephala*) and R4 (70% *P. maximum*, 30% *Gliricidia sepium*). Refusal and total feces were collected daily to calculate feed intake and digestibility. The eCH₄ emissions were measured using the GreenFeed® system (ID: 252, C-Lock, Inc., USA) every 4 hours, covering all hours of a day after 4 days. The results showed improved quality in supplemented diets compared to the control (R0). In steers, average voluntary intake was higher (25.08±1.39 g DM/kg BW) than in cows (21.36±1.9 g DM/kg BW). Intake was 24.13±4.09, 26.45±3.88, 24.74±2.8, 26.60±3.80 and 23.50±3.92 g DM/kg BW for R0, R1, R2, R3, and R4 respectively. In cows, it was 23.49±3.75, 21.58±3.73, 20.12±2.9, 22.77±4.71 and 18.84±2.85 g DM/kg BW respectively. Digestibility was 0.43±0.12, 0.53±0.1, 0.49±0.08, 0.42±0.08 and 0.44±0.12 for R0, R1, R2, R3, and R4 respectively. The eCH₄ emissions varied from one diet to another. Average daily production and eCH₄ yields in steers (0.83±0.14 g/kg BW; 33.51±4.19 g/kg DM intake) were higher than in cows (0.62±0.05 g/kg BW; 29.38±1.03 g/kg DM intake). In steers, there was no difference (p>0.05) between eCH₄ yields. The values induced by diets R0, R1, R2, R3 and R4 were 28.77±5.34, 28.24±9.07, 29.12±5.58, 30.85±11.76 and 29.94±8.07 g/kg DM intake in cows, respectively. In steers, these yields were 32.79±7.28, 29.73±7.91, 32.97±9.06, 40.64±10.77 and 31.43±6.65 g/kg DM intake, respectively. The eCH₄ yields recorded in this study varied according to the animal category and level of supplementation. They remain higher than the value of 23.3 g/kg DM intake reported by the IPCC (2019) and suggest that animal categories need to be considered to improve national greenhouse gas inventories in the livestock sector in SSA.

245 Effect of intake of *Leucaena leucocephala* on methane emissions and heat production of crossbred heifers

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Methane is a greenhouse gas with a greater global warming potential than that of carbon dioxide. Silvopastoral systems have been implemented in tropical regions in order to improve cattle productivity. *Leucaena leucocephala* is a legume with high nutritional value, however its inclusion in the diet is limited due to the presence of the non-protein amino acid mimosine. It has been reported that the inclusion of Leucaena in the diet of ruminants decrease the emission of enteric methane and it has been reported that the intake of mimosine decreased the concentration of thyroid hormones (triiodothyronine and thyroxine) in blood. Therefore, the aim of the present work was to assess the effect of consumption of a typical tropical diet with increasing levels of Leucaena on enteric methane emission, heat production and intake and digestibility of dry matter. Four crossbred (*Bos taurus* x *Bos indicus*) heifers fitted with rumen cannulas and weighing 435 ± 20 kg were assigned to a 4 x 4 Latin square design consisting of four periods and four levels of Leucaena inclusion: 0, 150, 300 and 450 g/ kg DMI. Each period lasted 18 days, with 14 days of adaptation and 5 days for digestibility and intake records and 4 days of measurement of gas exchange in two open circuit respiration chambers. On the last day of each period, blood samples were taken by venipuncture of the coccygeal vein to obtain blood serum and determine concentrations of thyroid hormones. The experimental ration was based on *Pennisetum purpureum* grass and increasing levels of Leucaena inclusion. Heifers were fed *ad libitum* daily at 08:00 h. In the respiration chambers, oxygen consumption, carbon dioxide and methane production were measured to estimate heat production. Data were analyzed using an analysis of variance with the R studio. Results showed that dry matter intake, apparent dry matter digestibility and thyroid hormones concentrations in serum were similar among treatments ($P = 0.72$; $P = 0.39$; $P = 0.69$, respectively). Dry matter intake and apparent dry matter digestibility were 7.95 kg DM/d and 560.9 g/kg DM, respectively. A reduction in methane emission and heat production was recorded ($P = 0.006$ and $P = 0.002$, respectively). These reductions were 56 g/d and 7.2 MJ/d when comparing the control treatment vs. the highest level of Leucaena inclusion. The results obtained demonstrate that it is possible to improve energetic efficiency of tropical livestock by including local forages of high nutritional value in the diet and at the same time reduce the emission of greenhouse gases. It is recommended for future research to quantify the complete energy balance to better understand changes in heat production.

171 Feeding fodder beet (*Beta vulgaris* L.) reduces methane emissions and nitrogen excretion from sheep

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Fodder beet (**FB**; *Beta vulgaris* L.) is increasingly utilised in ruminant production systems. Its high readily fermentable carbohydrate content and low crude protein (**CP**) content might reduce enteric methane (**CH₄**) emissions and nitrogen (**N**) excretion into the environment. This study aimed to investigate the effects of including FB in sheep diet on CH₄ emissions and N partitioning, compared with feeding traditional perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) based pasture. Twenty-four 12-month-old male sheep (liveweight 40±1.2 kg; mean±SD) were randomly allocated to two groups (FB vs. pasture) with 12 in each group and fed at 1.6 times their metabolisable energy requirements. Methane emissions were measured in two experimental periods using respiration chambers. The sheep in the FB group were fed 0.75 FB and 0.25 pasture on a dry matter (**DM**) basis in Period 1, while the diet was adjusted to 0.90 FB and 0.10 pasture in Period 2. The FB digestibility was 0.898±0.0034 (mean±SEM) for DM, 0.914±0.0035 for organic matter (**OM**), 0.647±0.0077 for CP, and 0.702±0.0082 for neutral detergent fibre, with digestible and metabolisable energy of 13.6 MJ/kg and 12.2 MJ/kg, respectively. All of these digestibility and energy values were higher than those in pasture ($P < 0.05$). Methane emissions from sheep fed a diet of 0.75 FB and 0.25 pasture were comparable to those from sheep fed only pasture, with CH₄ production at 17.2 vs. 18.1 g d⁻¹ and CH₄ yield at 18.6 vs. 19.6 g kg⁻¹ DM intake. However, when CH₄ emissions were expressed per unit of digestible DM or digestible OM, the inclusion of 0.75 FB resulted in a 13% to 16% reduction ($P < 0.05$). A more dramatic reduction in CH₄ yield, exceeding 60% ($P < 0.05$), was observed when FB constituted 0.90 of the diet. However, at this level of inclusion, the relative abundance of rumen bacteria associated with acidosis was increased. The 0.75 FB diet also led to decreased nitrogen intake and urinary N excretion, alongside a significant reduction in microbial CP production. Blood metabolic parameters revealed substantial changes, particularly in β -hydroxybutyrate and cholesterol levels. Fodder beet-fed sheep exhibited lower rumen pH and total short-chain fatty acids, with notable shifts in microbial communities, including an increase in *Sharpea* and a decrease in *Ruminococcus*. In conclusion, when sheep were fed a high proportion (0.75 or more) of FB in the diet, CH₄ emissions were significantly reduced and N excretion was also changed, with a notable decrease in urinary N. These findings suggest that FB can be a dietary component for mitigating the animal-level environmental impacts from sheep production, provided their protein requirements are met.

424 Does sward type influence lamb performance, enteric methane or the rumen microbiome in lambs post-weaning?

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Perennial ryegrass offers a unique economic advantage over alternative feedstuffs. However, its nutritive value exhibits seasonal variations which can hinder animal intake and performance. Legume and herb inclusion can boost sward quality in the latter half of the grazing season. Manipulation of the diet to reduce methane (**CH₄**) is an important strategy available, however those that require daily supplementation are not currently feasible in most pasture-based systems. Strategies to increase live-weight (**LW**) gain of animals at pasture, like sward diversification can reduce age at slaughter, decreasing the animal's lifetime emissions. This study assess the influence of sward type on performance, CH₄ and the rumen microbiome in lambs post-weaning. A randomized block design was employed to investigate five sward treatments: perennial ryegrass (**PRG**), PRG white clover (**PRG+WC**), PRG red clover (**PRG+RC**), PRG chicory (**PRG+Chic**) and PRG plantain (**PRG+Plan**). At weaning 120 lambs (n=24 per treatment) were selected in 2021 and 2022. Six CH₄ measurements were obtained per lamb between weaning and slaughter, using portable accumulation chambers. Rumen fluid was harvested using a transesophageal sampling-device. Companion forage inclusion, averaging 28% across sward types, enhanced sward digestibility and reduced fibre content. Data were analysed using PROC MIXED in SAS. Fixed effects of treatment, birth LS, reared LS, sex and dam parity were included, while dam was included as a random effect for all performance traits. Fixed effects of treatment, LW, time off feed and chamber were included for CH₄ g/day, CH₄ g/kg LW and CH₄ g/kg average daily gain (**ADG**). Contemporary group was included as a random effect for CH₄ traits. Statistical comparisons of the abundance of the rumen archaea were conducted on ASV with a mean abundance of >1% and also using PROC MIXED, with fixed effects of treatment, age and LW. Results show that lambs grazing PRG+WC, PRG+RC or PRG+Chic had a higher post-weaning ADG ($P<0.001$) of 29-60±0.7 g/day, compared to PRG lambs. Age at slaughter was reduced by 16-50±4.0 days ($P<0.001$) with companion forage inclusion. Lambs grazing PRG+WC (13.5 CH₄ g/day, 0.37 CH₄ g/kgLW, 93.7 CH₄ g/kgADG), PRG+RC (12.4 CH₄ g/day, 0.34 CH₄ g/kgLW, 80.5 CH₄ g/kgADG) or PRG+Plan (13.2 CH₄ g/day, 0.36 CH₄ g/kgLW, 101.5 CH₄ g/kgADG) ranked lower for CH₄ g/day (SEM±0.299; $P<0.001$), CH₄ g/kgLW (SEM±0.012; $P<0.001$) and CH₄ g/kgADG (SEM±4.11; $P<0.001$) compared to PRG lambs (14.3 CH₄ g/day, 0.40 CH₄ g/kgLW, 116.4 CH₄ g/kgADG). There was a lower abundance of *Methanobrevibacter* (SEM±1.2; $P<0.001$) and higher abundance of *Methanosphaera* (SEM±1.22; $P<0.001$) in lambs grazing PRG+RC than those grazing PRG. This study demonstrates that incorporating companion forages into perennial ryegrass swards enhances lamb performance, reducing age at slaughter, likely driven by superior sward quality. Additionally, findings indicate that diverse swards can achieve direct and indirect CH₄ reductions while manipulating the population of methanogens within the rumen, potentially offering a more favourable microbial signature to reduce CH₄ output.

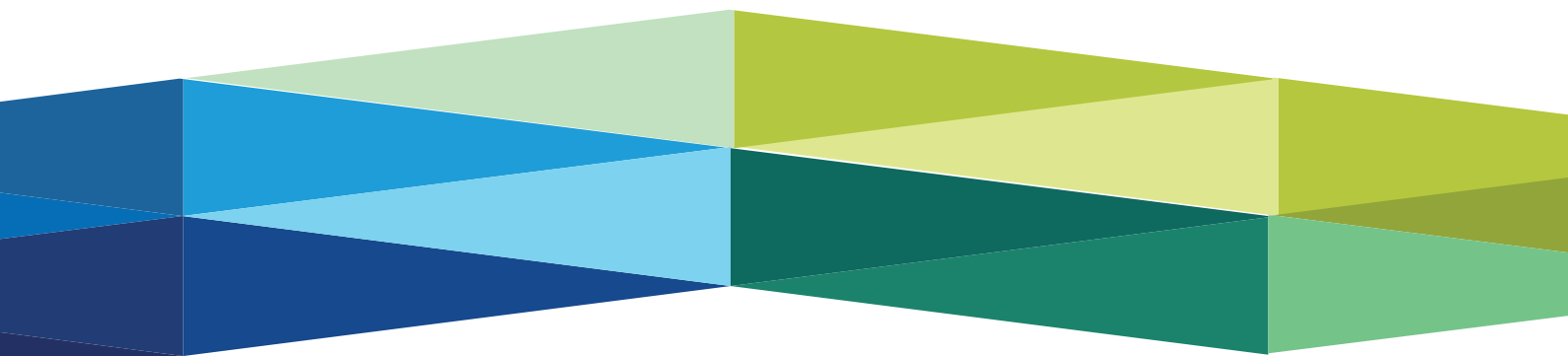


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Theme 2: Animal health, genetics, and genomics for climate resilience

2.1 Genetics meets climate action: Breeding strategies for low-emission livestock



436 Global Methane Genetics: acceleration of genetic progress for reduced methane emission in ruminants

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The Global Methane Genetics initiative aims to reduce methane emissions from the single largest emitter in the agricultural sector – ruminant livestock. The genetic differences in methane emission between animals can be identified and included in selection decisions to breed animals that emit less methane, whilst staying healthy and producing nutrient dense food. Genetic selection is a reliable, cost-effective, cumulative and permanent mitigation tool. Several countries have started recording individual methane emission in cattle and small ruminants on commercial farms allowing for estimation of breeding values for lower methane emissions. However, for routine implementation globally, the number of phenotypes is still too small and limited to few populations around the world.

The Global Methane Genetics initiative is a multi-million-dollar investment by the Global Methane Hub's Enteric Fermentation R&D Accelerator and The Bezos Earth Fund, coordinated by Wageningen University & Research. The overarching aim is to accelerate genetic progress towards low methane emitting ruminants. The primary goals in the first phase of the initiative are (1) to significantly expand recording of methane emissions in cattle and small ruminants, together with genotypes, pedigree and rumen microbiome information, (2) knowledge sharing and dissemination via global working groups, and (3) protocol and standard operating procedure developments by the ICAR Feed & Gas working group. The initial phase of the program consists of a portfolio of programs starting early 2025 and involving around 50 partners from academia, research organizations, cattle and small ruminant breeding organizations and other related stakeholders. The dairy cattle program reflects the major dairy breeds Holstein, Red dairy cattle, Jersey and Brown Swiss. The beef program is a multi-country effort around the world covering more than 15 beef breeds and crosses. One program is dedicated to Africa, focused on local breeds and crosses. A global sheep program covers more than 16,000 sheep in Europe, Australasia and Latin America. Complementing these programs is a global rumen microbiome initiative, which will establish the largest ever global metagenome reference population to determine a core metagenome that defines a high or low methane emission profile. Cattle and sheep genetic profiles for predisposition to host low rumen microbiome profiles will be determined and be used in breeding programs. This large-scale phenotyping and genotyping effort aims to result in more than 100,000 animals phenotyped and genotyped for methane emissions with sniffers, GreenFeed, portable accumulation chambers and laser methane detectors, and around 20,000 animals with rumen microbiome information in more than 25 countries in the Global North and South. A global database hosting phenotypes, genotypes, pedigree and microbiome information is currently being developed within the Global Methane Genetics initiative, enhancing data sharing across the world. In this presentation we will show the potential of genetic selection as a methane mitigation tool as will be implemented in the Global Methane Genetics initiative.

167 Denmark launches the world's first carbon tax – can cattle breeding mitigate methane emissions?

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To accelerate the green transition in agriculture, the Danish parliament has legislated the world's first carbon tax on livestock emissions, effective from 2030. The tax will initially be ~€40 per ton of CO₂e, increasing to ~€100 by 2035, with a deduction of 60%. Moreover, conventional Danish dairy farmers are from 2025 obliged by the Danish authorities to use feed additives like *Bovaer* or to increase the fat level in the diet in order to mitigate enteric methane emissions. At this, the costs from using feed additives are subsidized by the Danish state. This policy provides Danish dairy farmers with a strong financial incentive to reduce emissions. Moreover, it is in alignment with Arla's Incentive Model, which rewards sustainability efforts, but also introduce potential challenges with G×E interactions. Historically, Nordic cattle breeding has successfully improved productivity, fertility, and health through the Nordic Total Merit Index. These achievements have earlier raised the question: can cattle breeding also contribute to reducing enteric methane emissions and thereby lower farmers' carbon tax liability? And how will genetic models be affected by the use of feed additives on the large scale? This study explores the feasibility of establishing a genetic evaluation system tailored to Holstein and Jersey dairy breeds. We utilized the "sniffer" technique to measure individual enteric methane concentrations from commercial Danish herds equipped with automatic milking systems. The dataset was collected from 2020 to 2024 (not including use of feed additives) comprising 587,640 daily records from 4,019 Holstein cows and 231,377 records from 1,900 Jersey cows. Variance components were estimated using the AI-REML algorithm in DMU, applying a pedigree-based BLUP in a repeatability model. For Holstein cows, the estimated heritability and repeatability of methane concentration was 0.19 (SE: 0.03) and 0.35 (SE: 0.01), respectively, for primiparous cows and 0.16 (SE: 0.02) and 0.32 (SE: 0.01), respectively, for multiparous cows. For Jersey cows, the estimated heritability and repeatability of methane concentration was 0.27 (SE: 0.06) and 0.35 (SE: 0.02), respectively, for primiparous cows and 0.20 (SE: 0.03) and 0.38 (SE: 0.01), respectively, for multiparous cows. These estimates suggest that methane emissions are moderately heritable and repeatable, demonstrating the potential for genetic selection to reduce emissions. Based on these findings, Nordic Cattle Genetic Evaluation plans to introduce methane-specific breeding values in 2025, providing Danish dairy farmers with the opportunity to breed for more climate-friendly cattle. Genetic progress will thereby not only help to mitigate emissions, but also to decrease carbon tax costs, introduced as a climate action strategy, and being rewarded through sustainability programs such as the one from Arla. Yet, the dataset did not include the use of methane mitigating feed additives. However, future results using this data are expected to illuminate how the genetic model handles large-scale commercial use of feed additives on Danish dairy farms. This pioneering effort highlights the role of sustainable cattle breeding in addressing global climate challenges, offering a pathway for Danish dairy farming to contribute to the green transition in agriculture.

173 Transcriptome-enhanced genomic prediction models for methane emissions in sheep

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This study investigates the integration of tissue-specific RNA expression data to enhance genomic prediction models for methane emissions in sheep. Utilizing transcriptomic data from four distinct tissues – longissimus muscle, spleen, rumen papillae, and salivary gland – we analyzed 803 sheep between 2019-2021, with RNA sequencing performed on 48 individuals in 2021, examining over 20,000 genes per tissue. To address the high dimensionality of transcriptomic data, we employed principal component analysis (**PCA**) and systematically integrated principal components (**PCs**) as intermediate phenotypes into a Neural Network Genomic Best Linear Unbiased Prediction (**NNGBLUP**) model, benchmarking against a traditional GBLUP model with a baseline accuracy of 0.54. The integration of tissue-specific PCs revealed distinct patterns in predictive accuracy across tissues. The longissimus muscle showed strong performance, achieving a maximum accuracy of 0.623, though performance decreased with additional components. Salivary gland demonstrated stable predictive ability, reaching peak accuracy of 0.588 while maintaining consistent performance. Rumen papillae reached a maximum accuracy of 0.576, showing varying patterns of improvement. In contrast, spleen showed lower performance, achieving only 0.501 accuracy, suggesting limited utility for methane prediction. These findings show clear tissue-specific contributions to methane emission predictions. The variation in accuracy improvements across tissues indicates that each tissue contributes differently to prediction models. Notably, adding more components did not always improve accuracy, particularly evident in the longissimus muscle. The salivary gland's consistent performance suggests its reliability as a predictive marker. Our results show that most tissues supported increased genomic prediction accuracy compared to the baseline model, with longissimus muscle, salivary gland, and rumen papillae showing meaningful improvements. These insights advance our understanding of how different tissues contribute to methane emissions prediction and suggest focused approaches for improving breeding strategies. Based on these findings from a moderate-sized population, additional research with larger, genetically diverse populations would help validate these tissue-specific patterns and their potential use in breeding programs.

464 Scaling and measuring environmental impact mitigation through targeted breeding: A genetics-driven life cycle assessment

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Genetic improvement is responsible for significant gains in the productivity and efficiency of animal protein production and continues to be the most important driver for improving the sustainability of livestock systems. Targeted breeding programmes customized for regional value chains to improve environmental footprint and system profitability enable scalable, system-wide impact and can be an effective solution to meet the demand for sustainable beef. Structured breeding programmes for beef have not been widely adopted in much of the world, but the desire for more sustainable beef production is likely to provide incentives that will encourage producers to overcome previous barriers to adopting genetic improvement strategies. The incentive framework requires credible quantification of the effect of genetic improvement on the environmental footprint of beef production. Life cycle assessment (LCA) studies have previously demonstrated the relationship between increased performance efficiency and a reduction in environmental impact from beef systems, considering the full cycle from birth to consumption. However, these approaches are not well-suited for capturing the specific impact from targeted genetic improvement, nor are they capable of comparing rates of improvement from different programmes, which are both required for optimizing genetic decision-making for specific supply chain needs. Attributional LCA models were built for three beef x dairy production systems, each with unique product performance targets and distinct breeding goals and genetic inputs. A stepwise approach was developed to use commercial beef x dairy life cycle data to empirically determine the relationship between sire genetic merit, as measured by a bespoke terminal selection index for each system, and progeny performance for traits previously identified as being most relevant to environmental impact. Progeny performance was estimated for set levels of sire genetic merit and the environmental impact of progeny from sire genetics at the highest levels of merit were compared to progeny environmental impact from average sire genetics in each population, with particular focus on methane emissions from enteric fermentation, manure management and feed production. The results showed that total greenhouse gas emissions attributed directly to genetic inputs could be reduced by 1% to 10% per year, when using genetics targeted to the specific production system. This study demonstrated that the environmental impact of breeding programmes can be assessed using an LCA and was able to capture the added environmental benefits of implementing a targeted genetic improvement approach. The reductions from genetic improvement are incremental each year, but are cumulative and permanent over generations, and thus continued genetic gains will add to the size of the effect. Using an LCA approach that directly assesses and compares different genetic inputs enables the identification of breeding programme objectives that minimize environmental impact. Optimal targeted genetic interventions can be simultaneously implemented across many farms, scaling the aggregate environmental benefit and allowing for credible emissions reductions across the beef value chain.

206 To Develop and Incorporate a Carbon Sub Index into the Irish Sheep Breeding Program

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Breeding objectives are constantly updated to align with current economy, environmental concerns and market preferences. In a breeding objective, multiple traits are weighted by their economic value which is the value of a unit change in a particular trait, while holding other traits constant. The cost of production across all sheep farms have increased by 8% (Teagasc NFS, 2024), implying that, variable costs and sales prices used to calculate the current economic values are not reflection of current prices. Additionally, greenhouse gas (GHG) emissions from livestock are increasingly becoming a concern due to their effect on the climate change. Therefore, incorporating carbon into breeding objectives could mitigate GHG emissions through genetic improvement. The objectives of this study was to update the economic values for important traits included in the Irish sheep breeding objectives, and adding a carbon sub index. The Teagasc Lamb Production bio-economic model (TLPM) was used to update the economic values for traits of economic importance into four main sub-categories: production, maternal, lambing and health trait. The Teagasc sheep Life Cycle Assessment model was used to determine GHG emissions. A carbon value was defined as a change in total GHG emissions per unit change in the trait under investigation holding all other traits constant. The economic value of carbon was obtained using carbon trading value of €80/tonne CO₂ equivalent. The relative emphasis of each trait was derived using the selection index methodology. Results for economic values for each category were as follows; Economic values for maternal traits were; €56.72 per additional lamb born, – €0.59 per kg ewe mature weight (maintenance), and – €0.55 for barrenness (per %). The economic values for lambing traits were, €78.49 for lamb survival (per lamb), and – €0.35 to – €0.38 for direct lambing difficulty. Economic values for production traits were, –€0.39 for days to slaughter (per day), €3.57 per carcass conformation grade. The economic values for health traits were: – €0.31 for ewe lameness, – €0.39 for dag score, – €0.10 for lamb lameness, and – €0.21 for faecal egg count (per 50 eggs/g). The carbon economic value for the traits impacting GHG emissions were: €0.19 lamb surviving at birth, €20.08 number of lambs born, € 0.03 ewe mature weight (replacement), € 0.17 ewe mature weight (maintenance), € 0.09 days to slaughter (per day), € 0.15 carcass fat (per score). The relative emphasis for production, lambing, maternal, health and carbon indices were 50.13%, 36.56%, 0.00%, 3.35%, and 9.96% in terminal index and 15.45%, 11.66%, 53.91%, 1.11%, and 17.86% in replacement index, respectively. This study has shown that all the economic weights have increased in response to variable costs and prices. Positive economic values for carbon show that improving specific traits can increase GHG emissions. This presents a conflict between maximising productivity and minimising environmental impact. However, others can be complementary, suggesting it is possible to strike a balance between reducing emissions and enhancing profitability. Production traits continue to receive the highest emphasis, albeit reduced with the inclusion of the carbon index.

186 Breeding for low methane emitting dairy cows in the Netherlands

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Animal breeding is increasingly recognised as an effective strategy to reduce enteric methane (CH₄) emissions from ruminants. However, the lack of individual cow CH₄ emission recording has been a limitation to practical application. Consequently, implementation of CH₄ mitigation strategies in breeding programs are in their early stages of development. In the Netherlands, two successive projects between 2018 and 2025 aimed to collect a large number of enteric CH₄ records of individual cows that were recorded using 'sniffers' in the feed bin of milking robots. This resulted in a dataset with 74,569 weekly mean CH₄ concentration (ppm) records on 7,139 cows from 68 commercial dairy farms. Sniffers measure concentrations and in order to predict the breeding value for the total grams of CH₄ emitted by breath, an additional dataset was collected that included enteric CH₄ production (g/day) recorded by GreenFeed units. This dataset included 4,358 weekly mean records from 822 cows and 334 weekly means from 73 cows overlapped with both GF and sniffer records in the same period. Other animals were linked through (genomic) relationships, enabling the estimation of the genetic correlation. Several analyses have been performed on the datasets, with the following objectives: 1) define a CH₄ trait from the raw concentration measurements and estimate heritabilities and repeatabilities, 2) investigate the relationship between measurements by sniffers and GreenFeed units, 3) investigate the relationships between CH₄ and other breeding goal traits, and 4) investigate the expected impact of breeding for low CH₄ emissions. The defined phenotype for weekly mean CH₄ concentration measured by sniffers had a moderate heritability of 0.17 ± 0.04 and a repeatability of 0.56 ± 0.03 . The genetic correlation between measurements by sniffer and by GreenFeed units was 0.76 ± 0.15 , indicating that selection for lower CH₄ concentrations will result in a reduction of total CH₄ production output in g/day. The genetic relationships among CH₄ concentration, DMI, body weight, and milk yield traits were weak: 0.06 ± 0.10 with dry matter intake, -0.04 ± 0.10 with body weight, and -0.04 ± 0.08 with milk yield for first parity cows. Selection index calculations showed that with weak genetic correlations with other breeding goal traits, a large selective weight can be put on mitigating CH₄ while continuing to improve other important breeding goal traits including health and fertility. Thereby, reducing enteric CH₄ by 1% per year in the population through animal breeding is feasible. This dataset will be the basis for national breeding value estimations for enteric CH₄ of Holstein dairy cows, which will be published in early 2025. Here, the breeding goal trait will be CH₄ production (g/day), as recorded by GreenFeed units, to which sniffer concentration (ppm) measurements serve as a proxy. Ongoing research will focus on estimating genetic correlations between GreenFeed recorded CH₄ production and other breeding goal traits, to ensure effective integration of CH₄ concentration and production phenotypes in selection indices that aim to mitigate CH₄ emissions. In addition, milk mid-infrared spectra will be investigated to be used as an additional proxy for CH₄ production.

280 Genomic evaluation of methane emissions and feed efficiency in Holstein bulls

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Climate change poses a critical global challenge, with the livestock sector contributing significantly to greenhouse gas (**GHG**) emissions, particularly methane (**CH₄**) and carbon dioxide (**CO₂**). Dairy cattle, as a major source of enteric methane, play a pivotal role in the environmental footprint of agriculture. A potential method to reduce CH₄ emissions in dairy cattle is through genetic selection. Based on a paper from 1992 (Nieuwhof et al., 1992) there is indication that measuring and breeding for traits in dairy bulls can have a direct impact on the phenotypic expression of the same trait in their progeny. Since then, little has been done to test the validity of environmental traits measured in Holstein bulls. Hence, the objective was to measure and evaluate the heritability of CH₄ (g/day), feed intake (g/day), and live weight (kg) in growing Holstein bulls between March 2018 and November 2024. A Greenfeed system collected a total of 3,578 valid CH₄ and CO₂ records from 194 bulls. Feed intake was recorded using five Roughage Intake Control (**RIC**) systems, capturing 8,307 feed intake records from 258 bulls. 2,748 live weight records were collected from 586 bulls in the same time span. Bulls were divided into contemporary and pen groups based on their time of arrival at the genetic center. A single trait ssGBLUP model was developed and tested in BLUPf90 to estimate heritability for CH₄, feed intake, and live weight as 0.29, 0.30, and 0.28, respectively. A third measure, “feed saved while growing,” was obtained by correcting the GEBV for feed intake with live weight. The moderate heritability shown by methane and the growing traits underscores the potential for genetic selection to reduce the environmental impact of dairy cattle.

104 Evaluating genetic and non-genetic influences on predicted methane emission traits in South African Holstein cows

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Mitigating methane emissions through genetic selection in dairy cattle is imperative to ensure environmental and the industry's long-term sustainability. Unfortunately, lack of enteric methane production data in South Africa, to compute methane yield and intensity, presents a significant challenge to selection for methane reductions in dairy cows. To overcome this, methane production may be predicted from its proxy traits, using the available IPCC prediction equations. There is, however, scarcity of knowledge on genetic and non-genetic aspects of the predicted methane emission traits, to determine if they can serve as a useful selection criteria for reducing enteric methane emissions in South African dairy cattle. Therefore, this study was carried out to determine non-genetic factors affecting predicted methane emission traits, followed by estimating their heritabilities and genetic correlation between them in South African Holstein cows. A total of 595 test-day records of 132 Holstein cows were used to predict daily dry matter intake (kg/day) from milk yield, butterfat content, cow weight and a number of week of lactation. The animals formed part of an extensively-fed herd in Mpumalanga province of South Africa. The data were for the first four parities for animals that calved between 2013 and 2023, and were participating in the National Milk Recording and Improvement Scheme. The predicted methane production was calculated using the IPCC Tier 2 approach, whereas predicted methane intensity (g methane/kg energy-corrected milk) was calculated from predicted methane production and energy-corrected milk. An analysis of variance was performed to test the effects of parity-test-date, lactation stage, age of cow at calving, and body condition score on the predicted methane traits, using the Generalised Linear Models procedure of the Statistical Analysis System. These effects were fitted into the bivariate repeatability animal models to estimate heritabilities and genetic correlations using the AIREMLF90 program, where the random effects were the animal additive genetic, permanent environment, and residual effects. All the tested effects significantly influenced ($P < 0.05$) predicted methane intensity and production, except body condition score ($P > 0.05$) on latter trait. Predicted methane production means were significantly lower ($P < 0.05$) in early lactation (339.32 ± 6.02) than in mid (350.58 ± 5.78) and late lactation (348.29 ± 5.83 g/day). The means for predicted methane intensity significantly increased ($P < 0.05$) with lactation stage, ranging from 17.29 ± 0.42 in early lactation to 20.28 ± 0.41 g/kg in late lactation. Heritability estimates were moderate for predicted methane production (0.24 ± 0.01) and low for methane intensity (0.07 ± 0.01). The genetic correlation between these traits was negative and moderate (-0.53 ± 0.07). Parity-test-date, lactation stage, and age of cow at calving are significant sources of variation in predicted methane production and intensity, and should, therefore, be accounted for in genetic evaluation models. Results exhibited modest heritable variation in predicted methane production and intensity, suggesting a scope to reduce methane emissions through selection in South African Holstein cattle. Moreover, medium genetic correlation between predicted methane production and intensity, may be used to improve accuracy of selection in multi-trait analyses for methane emission reductions in dairy cattle.

335 Using milk and rumen phenotypes as a proxy for enteric methane emissions in lactating ewes

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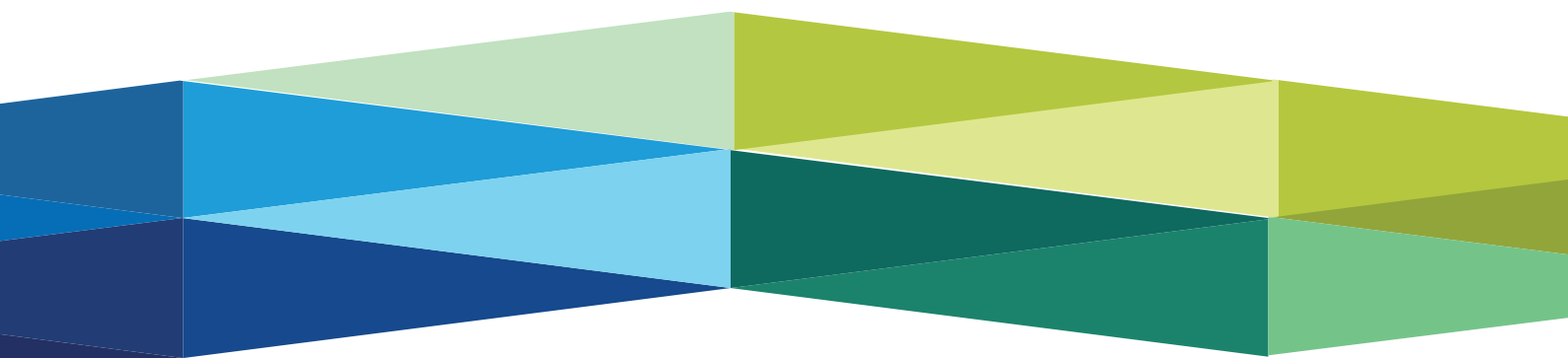
Genomic selection can be used to breed for ruminants that emit less methane but requires on-farm measurements of methane. However, existing measurement technologies have several limitations that means that they may not be suitable for lactating animals. Enteric methane is closely linked to the microbial fermentation of feed in the rumen, which in turn impacts nutrient availability for downstream processes such as tissue deposition and milk production. This suggests that breeding for low methane emitting ruminants could alter the composition of the milk and rumen microbial profiles. If this is the case, associated changes in milk composition may provide proxy measures of methane for use in selective breeding of low methane emitting ruminants. In this study, we investigated the impact of breeding sheep for divergent methane yield has on rumen and milk composition, and the potential of using differences in milk composition as a proxy measure of methane emissions in lactating ewes. Rumen fluid and milk samples were collected at approximately six weeks post lambing from 400 genotyped lactating ewes from a sheep research flock bred specifically for high and low methane emissions. Methane was measured using portable accumulation chambers (**PAC**). Volatile fatty acid (**VFA**) and metagenomic microbiome community profiles were generated from the rumen samples and fatty acid (**FA**) profiles and mid-infrared (**MIR**) spectra were generated from the milk samples. No differences in total fat, protein and lactose percentages in the milk were observed but the milk FA profiles differed between methane selection lines, with higher levels of polyunsaturated FA and branched-chain FA and lower total saturated FA contents in ewes from the low methane line. Higher proportions of acetate relative to propionate were observed in the rumen VFA profiles for the low methane ewes. Methane was predicted from the rumen VFA and metagenomic profiles, and the milk FA and MIR profiles, which formed the proxy methane traits. The heritabilities of these proxies were between 0.12 to 0.36 and correlated (between 0.29 and 0.42) with methane measured using PAC. The genetic correlation between proxies and PAC methane was between 0.52 and 0.71, and the estimated efficiency of indirect selection for PAC methane was higher for the milk sample proxies (49-75%) than the rumen metagenomic profiles (45-47%) and rumen VFA profiles (12-38%). Hence, milk FA, MIR and rumen microbial composition phenotypes have the potential to be used as proxy measures of methane in lactating ruminants. These results suggest that the number of animals with predicted methane emissions could be increased manifold using proxy traits. This, in particular, would facilitate selection for lowered enteric methane emissions in systems and countries with limited infrastructure.



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2.2 Breeding resilience: Integrating emissions, health and environmental adaptation



261 Methane emissions differ in the daughters of sires with high or low methane breeding values.

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Ruminant animals are a major source of greenhouse gas emissions. Selection for reduced emissions is likely possible, due to genetic variation between animals. It is challenging to measure methane in grazing dairy systems, such as those used in New Zealand. Furthermore, dairy genetic evaluation systems typically use daughter performance to estimate a bull's genetic merit for a trait. However, methane is a trait that can be measured on the bull. Since 2021 New Zealand's largest two breeding companies have measured methane emissions and feed intakes in young dairy sires.

A validation study to confirm that bulls were a suitable phenotyping model for lactating cows began in 2022. 486 bulls had been measured at this point, and the top 25 and bottom 25 bulls were selected as sires based on their methane breeding value (**BV**), which was calculated on total methane emissions of each bull, adjusted for the genetic variation in feed intake. A single 2000 cow herd was inseminated. The resulting female progeny were DNA matched to sire and reared on-farm.

The resulting 397 heifers were allocated to four groups based on liveweight and measured for 39 days while they were between 9 and 15 months old. Lucerne hay cubes were fed *ad libitum* and intake measured (RIC2 Discover Feed bins), following a week of increasing allocations in paddock. Methane emissions were measured via GreenFeed, with up to 5 visits per day allowed. Concentrate pellets were used as a bait feed, with animals who visited 5 times a day consuming around 0.95 kg of pellets daily. All data from the first seven days of the trial was excluded. Analyses were undertaken in SAS using a repeated measures model. Daughters of sires with a high methane BV were denoted as HIGH, and daughters of low methane BV sires as LOW.

Methane emissions were greater for HIGH heifers than LOW heifers (173 and 168 g/day; $P < 0.05$). Although emissions increased over the trial ($P < 0.001$), there was no group x week interaction ($P = 0.21$). Feed intake also increased over the trial ($P < 0.001$), but was not different between the HIGH and LOW daughters (9.5 and 9.6 kg DM/day; $P = 0.27$). Methane yield (g/kg DM) was greater for HIGH heifers than LOW heifers (18.5 and 17.7 g/kg DM per day, $P < 0.001$). Yield decreased during the trial ($P < 0.001$) with no week x group interaction ($P = 0.52$). These results confirm that methane emissions of daughters of LOW methane BV sires are lower than daughters of HIGH methane BV sires. The similarity in feed intake between the HIGH and LOW daughters suggests that selection for lower emissions is possible, without impacting feed intake. The next phase of this research will investigate whether the difference in emissions of the HIGH and LOW daughters is maintained during their first lactation. The outcome of this investigation will confirm whether young bulls are a suitable phenotyping model for methane emissions in lactating dairy cattle.

459 Methane production in mid-lactation Holstein cows: genetic parameters and spectra-based predictions

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Enteric methane (**CH₄**) is a major source of greenhouse gas emissions from dairy farming. Routine recording of CH₄ emissions at commercial farms can be challenging and costly. Fourier-transform mid-infrared (**FT-MIR**) spectroscopy is a powerful phenotyping tool that offers high-throughput, low-cost, non-invasive, and real-time predictions. The goal of this study was to estimate relevant genetic parameters for CH₄ emission traits and evaluate the feasibility of predicting CH₄ production using spectra data. We used data from 1,171 mid-lactation Holstein cows collected since June 2023. Data consisted of daily CH₄ production (**MeP**, measured using GreenFeed), daily dry matter intake (**DMI**), daily milk yield (**MY**), milk composition and milk spectra data (2 to 4 times/wk), and body weight (**BW**) records (every 2 to 3 wk). We calculated two residual methane traits: 1) residual methane intensity (**RMI**), defined as MeP regressed on milk energy and metabolic BW, and 2) residual methane yield (**RMY**), defined as MeP regressed on DMI. All cows were genotyped with 68,472 SNP. Milk spectra data consisted of FT-MIR absorbances at 1,060 data points in the infrared wavenumber range from 5,008 to 925 cm⁻¹. Interestingly, both RMI and RMY traits exhibited large variability: RMI ranged from -147 to 162 g/d, and RMY ranged from -146 to 138 g/d. The heritability of MeP, RMI, and RMY were 0.36±0.07, 0.24±0.06, and 0.28±0.08, respectively. Furthermore, we evaluated three alternative models to predict CH₄ production using a Bayesian approach: model XB with cohort (36 levels combining trial and treatment), days in milk (50-200), MY, and BW^{0.75} as predictors; model S as model XB plus FT-MIR data; and model GS as model S plus genomic data. Model predictive ability was evaluated in cross-validation using 10-fold cross-validation (**cv105**) and leave-one-trial-out cross-validation (**loto**). Model GS achieved the highest correlations between the predicted and observed CH₄ production (cv105: 0.86 and loto: 0.77), followed by model S (cv105: 0.72 and loto: 0.54) and model XB (cv105: 0.61 and loto: 0.33). Our findings suggest a promising genomic evaluation for methane emissions in US Holstein cows and also provide strong evidence that supports the use of genomics and milk spectra to predict methane emissions in dairy cows.

127 Contributions of community-based breeding program for reduced greenhouse gas emissions in Menz sheep

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Community-based breeding programs focusing on local genotypes have been promoted as a strategy for the genetic improvement of small ruminants in Ethiopia. These programs are designed for low-input systems and consider farmers' needs, views, decisions, and active participation, from planning to implementation. The objective of this study was to estimate greenhouse gas emissions and evaluate environmental trade-offs of production interventions from Menz sheep community-based breeding program implementing villages. Primary data collected for 15 years, since the start in 2009 to 2023, was used. A total of 25,553 and 20,194 data points on growth and reproduction related traits, respectively, collected from 450 participant farmers were used for the study. Common animal feeds and manure management practices of the farm households were collected from the literature and used as input for greenhouse gas estimations. The greenhouse gas emission was analyzed with the Global Livestock Environmental Assessment Model interactive (**GLEAM-I** Version 1.9). The software estimates the greenhouse gases from the animal (herd module), feed types (feed module), and manure disposal (manure module). To see the contribution of the animal selection program on greenhouse gas emissions, values for feed and manure modules were used constantly throughout the selection years. The overall mean greenhouse gas emission produced from Menz sheep community-based breeding program was 336.73 ± 71.42 metric tons of carbon dioxide equivalent (**MtCO₂-eq**) and has an increasing trend by 5.79 Mt CO₂-eq across selection years. The protein production was 749.23 ± 209.85 kg year⁻¹ with 31.52 kg increment across the selection years. The overall mean emission intensity was 0.46 ± 0.07 Mt CO₂-eq year⁻¹, which shows a 0.012 Mt CO₂-eq emission reduction across selection years. The total greenhouse gas emissions and protein production have increased by 11% and 6%, respectively, while the emission intensity decreased by 7% across the selection years. The emission intensity demonstrates a fluctuating decline trend across the selection years, which is related to differing selection responses for the target traits used for greenhouse gas emissions. From greenhouse gases, methane accounts for about 95% of the total gases, followed by nitrous oxide (4%) and carbon dioxide (1%). Of the methane gas produced, 80.09% is sourced from enteric fermentation. Beyond the positive response of selection, the contributing factor for the decreasing trend of the emission intensity is related to the presence of integrative interventions, such as animal health, feed and feeding, and early age marketing. This indicated the presence of a positive correlation (synergy) between the community-based breeding program interventions environmental footprint. The environmental trade-off related to the community-based breeding program resulted in increased animal performance (protein production) and reduced environmental footprints through decreased emission intensity. The result provides insight for breeders, policymakers, and organizations working on the livestock sector targeting to lowered environmental burden through different interventions. Besides, it is imperative to estimate greenhouse gas emissions from other sheep community-based breeding program implementing villages to optimize the breeding program towards raising low-emission and environmentally adaptive breeding programs.

123 Beyond Animal Health: How Gastrointestinal Nematodes Shape Productivity and Greenhouse Gas Emissions in Sheep – A Meta-Analysis

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Livestock diseases are a major constraint to sheep productivity and health, reducing feed intake, nutrient use efficiency, and overall performance. At the same time, livestock production contributes significantly to greenhouse gas (**GHG**) emissions, with enteric methane being a primary source. Among livestock diseases, gastrointestinal worms pose a particularly severe challenge in sheep production, impacting the gastrointestinal tract and potentially influencing enteric methane emissions. Despite their importance, research into the relationship between gastrointestinal worm infections and methane emissions is limited and has yielded variable results. To address this knowledge gap, this study employed a meta-analytic approach to synthesize existing data and evaluate the impact of gastrointestinal worm infections on sheep methane emissions.

Data from sheep experiments investigating the effects of gastrointestinal worm infections on enteric methane (**CH₄**) emissions were consolidated into two datasets. The first dataset included eight studies comprising ten experiments that directly measured enteric CH₄ emissions in infected and uninfected sheep using respiration chambers. The second dataset utilized 19 experiments that reported dry matter intake (**DMI**) and liveweight gain (**LWG**) data for infected and uninfected groups.

Using DMI data, enteric CH₄ production (g CH₄/day) was estimated through regression equations. Methane emission intensity (g CH₄/kg LWG) was calculated by combining estimated CH₄ production with reported LWG data. A meta-analysis was conducted to calculate the relative mean difference (relative **MD**) between infected and uninfected groups for daily CH₄ production, CH₄ yield, and CH₄ intensity.

Gastrointestinal worm infections did not significantly affect daily CH₄ production (g CH₄/day) or CH₄ yield (g CH₄/kg DMI) ($P > 0.05$). However, infected sheep exhibited a numerical increase in CH₄ emissions, with a relative MD of 18.3 % for daily CH₄ production and 12.5 % for CH₄ yield. Additionally, infected sheep showed a tendency for higher CH₄ intensity, with a relative MD of 19.2 % ($P = 0.07$).

This study found no statistically significant effect of gastrointestinal worm infections on absolute CH₄ emissions or yield. However, the results suggest that infections increase CH₄ emissions per unit of product, as indicated by CH₄ intensity. The limited number of studies included in this analysis highlights the need for a larger dataset to provide more robust and definitive insights.

These findings emphasize the dual role of gastrointestinal worms as a risk factor for both reduced productivity and increased GHG emissions in sheep production. Effective management of gastrointestinal worms could enhance animal welfare, boost food security, and reduce the environmental footprint of sheep production systems, contributing to more sustainable livestock management practices.

322 Repeatability of methane traits from Greenfeed & their longitudinal associations with feed efficiency in Nordic RedCattle

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Mitigation of methane (**CH₄**) from the livestock production systems has been one priority areas by many countries in particular in those with carbon neutrality commitments. Apart from feed additives and other nutritional and managemental strategies, identification, and selection of animals with better feed utilization efficacy (**FE**) is suggested as one of the most sustainable strategies to lower the carbon footprint of milk production in dairy cows. The success in this strategy depends largely on the type of traits considered and the magnitude of associations between them. Unfortunately, it still remains to be clarified how CH₄ emission and the different FE metrics are related to each other, which will require direct and accurate measurements of these traits on large numbers of animals and such information particularly for the Nordic Red cattle is required. The aims of this study were first to estimate animal correlations between CH₄ output and feed efficiency traits at different stages of lactation and secondly to evaluate the effects of selecting on alternative FE metrics on CH₄ emission. Data was from 170 primiparous Nordic Red dairy cows of Luke's Jokioinen research farm and included 60,000 repeated records. CH₄ and CO₂ were measured using two GreenFeed Emissions Monitoring (**GEM**) units. Methane output traits included CH₄ production (**MeP**=CH₄ g/day), CH₄ yield (**MeY** = g CH₄/kg DMI), CH₄ intensity (**MeI**= g CH₄/kg ECM) and residual CH₄ production (**RMP**) and were merged with FE traits: residual feed intake (**RFI**), residual carbon dioxide (**RCO₂**) and residual milk yield (**RMY**). FE traits were calculated as a difference between actual and predicted DMI, CO₂ production, and milk yield, respectively. Univariate and bivariate random regression models were used to estimate correlations within each trait and between CH₄ and FE traits, respectively fitting models that included fixed effects of year*month, test-date, fixed regressions, and random animal and residual effects. Over lactation the repeatability of MeP, MeI, MeY and RMP traits on daily average data ranged from 0.35 to 0.48, 0.39 to 0.54, 0.35 to 0.53 and 0.32 to 0.46, respectively. Correlations within traits, between different days in milk (**DIM**), ranged from 0.29 to 0.94, 0.46 to 0.95, 0.34 to 0.95, 0.16 to 0.93 (with $\pm$ SE ranged from 0.01-0.04), respectively for MeP, MeI, MeY and RMP. For CH₄ traits, animal correlations between mid-lactation and all other days ranged from 0.78 to 0.98 ($\pm$ SE = 0.01-0.03) indicating that this stage of lactation may be the best time to take representative measurements of CH₄ emission. The overall across lactation animal correlations between CH₄ and FE traits were 0.2, 0.6, and 0.42 between MeP and RFI, MeP and RCO₂, and MeP and RMY, respectively with $\pm$ SE ranged from 0.03-0.08. Ranking top cows on the basis of bivariate solutions indicated that using RCO₂ trait to select animals will have a better potential to improve FE as well as lower methane emissions than the other alternative FE metrics.

314 Associations between energy efficiency and methane production in dual-purpose Fleckvieh cows

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Enteric methane production is an important source of greenhouse gas emissions and also represents an energy loss for the cow. The relationship between methane production and energy efficiency has been studied primarily for Holstein cattle, with inconclusive results. This raises the question of whether improving either methane production or energy efficiency through selective breeding will also improve the other trait. The objective of this study was to assess the phenotypic relationship between methane production and different energy efficiency traits in Austrian dual-purpose Fleckvieh cattle. Data were recorded at the Federal Agricultural Research Centre in Raumberg-Gumpenstein between September 2023 and April 2024. The GreenFeed System (C-Lock, Inc.) was used to measure daily methane production. Daily feed intake data were collected through Calan gates (American Calan Inc.). Milk yield and body weight were recorded daily, and milk samples were collected twice weekly for each milking on the test day. Data from 20 cows were available for the analyses. Two traits have been defined to assess energy efficiency: net energy efficiency (**NEE**) and residual energy intake (**REI**). NEE was calculated as the ratio of milk energy to energy intake corrected for energy sinks (i.e., metabolic body weight, body weight changes). REI was defined as the difference between actual and predicted energy intake. For the prediction of the energy intake, the same energy sinks were used as for NEE. Pearson correlations were used to analyse the associations between methane production and energy efficiency traits. The correlations between methane production and the two energy efficiency traits were very weak ($r = -0.021$ for NEE and $r = 0.037$ for REI) and not statistically significant ($P = 0.78$ for NEE and $P = 0.63$ for REI). This suggests that for the analysed dataset there is almost no phenotypic relationship between them. However, further analyses are needed and will be included in the overall project of this study, focusing on genetic analyses of these and related traits.

114 Multimodal data-driven insights into cattle behaviour for adapting livestock management to climate challenges

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Climate change poses significant challenges to livestock management, particularly in regions grappling with rising temperatures and variable weather patterns. Understanding how livestock adapt their behaviour to these evolving conditions is essential for enhancing climate resilience in grazing systems. In this work, we combine climate and behavioural data to develop novel measures that help assess cattle responses to climate variability, offering insights to improve herd management strategies. We utilize data collected via wearable sensing technologies to predict cattle behaviour and track their positions. The wearable device used is the eGrazor collar, developed by CSIRO, which incorporates a triaxial accelerometer sensor and a GNSS receiver to capture high-resolution data on cattle location, movement, and behaviour. Trials conducted in Queensland (November-December 2022 and 2023) involved 60 Brahman and Droughtmaster cattle. The collected data was analysed to explore how cattle adapt their behaviour patterns, including grazing, ruminating, resting, walking, and drinking, to temperature fluctuations and environmental stressors. The findings demonstrate potential for forecasting cattle behaviour in different climate scenarios based on accelerometer data. However, the results also highlight the need for additional research to better capture cattle responses to climate stressors and link behavioural changes to productivity and welfare outcomes. Therefore, we expand our approach to a multimodal framework integrating GNSS data of the cattle and environmental data of the pasture. Using satellite imagery, we identified shaded and open areas, enabling calculation of the time each cattle spent in shade versus under sun. An additional measure included is the distance travelled by cattle, providing information on how cattle interact with their environment to mitigate heat stress. We also utilized behavioural profiles segmented by day and night to assess temporal shifts in behaviour patterns. The developed multimodal approach offers a robust set of measures that effectively capture cattle behavioural adaptations to climatic conditions. Preliminary correlation analysis revealed that during days of higher CCI, cattle increased time in the shade, reduced daytime grazing, shifted grazing to nighttime, and prolonged daytime resting. By incorporating multimodal data into predictive models, such as generative models, we aim to enhance prediction of cattle responses under future climate scenarios. Ultimately, the proposed approach provides actionable insights for livestock management in a changing climate and identifying more resilient animals, equipping livestock farmers with tools to improve herd resilience and productivity.

118 Association between meteorological variables and milk yield traits in crossbred dairy cattle under subtropical climate

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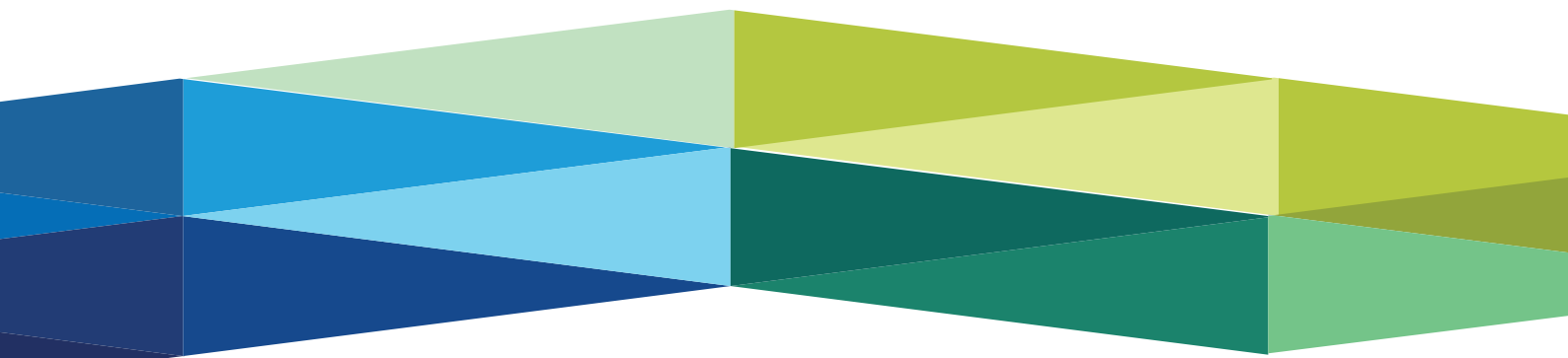
The study was conducted on crossbred cattle maintained at Directorate Livestock Farms (**DLF**) of Guru Angad Dev Veterinary and Animal Sciences University (**GADVASU**) in Ludhiana, Punjab, India to assess the influences of meteorological variables on milk yield traits of crossbred cattle. Data on milk yield traits and the meteorological variables were collected for a period from 1991–2018. General Linear Model was applied to assess the influences of meteorological variables and other fixed factors on daily milk yield. Simple linear regression models were fitted to analyze the effect of meteorological variables on other milk yield traits. The results showed that the effects of Tem, THI and ATHI on DMY were found to be significant ($p < 0.01$). The effects of Tem, THI and ATHI on TLMY, 305_DMY, LL and DPY were negative and non-significant, while negative and significant ($p < 0.01$) on PY. The average 305_DMY decreased by 8.68, 5.72 and 5.59 kg per a unit increase in Tem, THI, and ATHI values, respectively. The average LL also showed a decline by 0.11, 0.11, and 0.13 days for per unit rise Tem, THI, and ATI correspondingly. From April-October, climate variables increased beyond the thermal thresholds (21°C for Tem, 75% for Hum, 68 units for THI, 316 W/m² for SR and 71 units for ATHI) for daily milk production in crossbred dairy cattle. The animals experienced heat stress for seven months (April-October) leads to reduction of milk production and may be controlled by heat stress management at the farm.



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Theme 3: Rumen microbial genomics and nutrition



96 Identification of novel rumen microbial strains for enhanced feed efficiency and methane reduction

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The rumen microbial community plays a crucial role in digestion and nutrient absorption in livestock. However, underexplored microbial strains may have untapped potential to improve feed efficiency and reduce methane emissions. This study investigates the role of novel rumen microbial strains in optimizing livestock nutrition and sustainability.

Rumen fluid samples were collected from 30 cattle, divided into two groups based on dietary regimes: high-fiber and high-concentrate diets. DNA extraction was followed by 16S rRNA gene sequencing and metagenomic sequencing using Illumina technology. Bioinformatic analysis was performed using QIIME 2 for community profiling, and functional gene analysis was conducted using MetagenomeSeq to identify genes involved in fiber degradation, SCFA production, and methane reduction. Statistical analyses were performed in R, including alpha diversity (Shannon index), beta diversity (Bray-Curtis dissimilarity), and correlations between microbial abundance and feed efficiency using Pearson's correlation. Differences in microbial abundance between diet groups were tested using the Kruskal-Wallis test.

Metagenomic analysis revealed a significant difference in microbial composition between the diet groups ($p < 0.05$), with high-fiber-fed cattle exhibiting higher microbial diversity (Shannon index: 3.4 vs. 2.8). Novel strains, including *Fibrobacter succinogenes* and *Methanobrevibacter smithii*, were identified, with a positive correlation between the abundance of these strains and improved feed efficiency ($r = 0.72$, $p < 0.01$). Functional gene analysis revealed that these strains possessed genes for enhanced fiber degradation (cellulases, xylanases) and SCFA production (acetate, propionate). Furthermore, *M. smithii* was associated with a 25% reduction in methane production ($p < 0.05$). Strains linked to methane reduction were more abundant in the high-fiber group ($p < 0.01$).

This study identifies novel rumen microbial strains that contribute to enhanced feed efficiency and reduced methane emissions. The significant correlation between microbial abundance and feed efficiency highlights their potential for improving livestock nutrition. Additionally, the presence of methane-reducing strains suggests an opportunity for mitigating the environmental impact of livestock farming. Future studies will focus on isolating and testing these strains for in vivo applications to further optimize livestock productivity and sustainability.

358 The RUMEN Gateway Project: improving culturability of the microbiota to innovate global ruminant production systems

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The RUMEN Gateway Project is an international research initiative officially launched in October 2023 and led by Queen's University Belfast. It builds on the success of the Hungate1000 project and aims to advance our understanding of the rumen's thus far uncultured microbiota and its potential to drive innovation in livestock systems. This initiative brings together partners from 14 countries across the Global North and South, with new collaborations and institutional partnerships continuously being established. These efforts are further strengthened by the recent establishment of a comprehensive collaboration agreement amongst the institutions involved, emphasizing the project's global significance and commitment to addressing one of the most pressing challenges in greenhouse gas mitigation – methane emissions from livestock. This is a flagship project of the Global Research Alliance on Agricultural Greenhouse Gases, funded by the Global Methane Hub. It focuses on uncovering the complex dynamics within the rumen microbiome, a critical component of ruminant digestion, by isolating previously uncultured microorganisms and conducting extensive phenotyping and genotyping analyses of pure and co-cultures recently isolated from the rumen. These efforts aim to broaden our understanding of ruminal fermentation processes, including enteric methane generation, and support the development of innovative technologies to reduce global methane emissions, such as small molecule inhibitors, direct-fed microbials and vaccines targeting methanogenic archaea. A distinguishing aspect of the RUMEN Gateway Project is the adoption of diverse culturomics approaches across partner institutions, with experts specialising in archaea, bacteria, fungi, phage, and protozoa working collaboratively to apply tailored methodologies that ensure comprehensive coverage of the rumen microbiome, enabling an integrated exploration of its diversity. The conventional and high-throughput anaerobic culturing methodologies employ both rich and selective media types with the objective of optimising the recovery of previously uncultured microorganisms. Through

this collaboration, the project seeks to establish the world's most comprehensive open-access collection of rumen microorganisms, encompassing a diverse range of samples from different ruminant species, regions, and diets. As part of this collaborative strategy, the US Department of Energy's Joint Genome Institute (**JGI**) plays a pivotal role, in supporting the RUMEN Gateway Project by providing essential genomic and bioinformatic resources. By addressing the significant challenges of isolating anaerobic rumen microbes *in vitro*, this project expands the diversity of cultured isolates and provides new insights into microbial evolution, ecosystem functions, and sustainable environmental practices. To date, a biobank of 726 bacterial isolates has been established, with ~100 identified as novel. DNA extraction and *in vitro* phenotyping, including assessment of nutritional requirements and VFA production profiles, are currently in progress. The knowledge generated will contribute to refining global climate models and advancing our understanding of global nutrient cycling. In conclusion, the RUMEN Gateway Project plays a pivotal role in advancing our understanding of the complex rumen microbiome. Collaboration across multidisciplinary projects, as well as the generation of open-access genomic data and compatible databases will provide essential insights that drive environmental sustainability. Beyond its immediate applications, the findings are expected to inform biotechnological advancements and improve the resilience and sustainability of ecosystems worldwide.

89 3-Nitropropionic acid enhances hydrogen utilizing microbial communities during rumen fermentation in vitro

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During anaerobic fermentation in ruminants, each elementary step in the conversion of feed into nutrients, requires the interspecies hydrogen transfer between molecular hydrogen (H_2) producers and consumers. In this study, we utilized a potent methane reducing nitro compound and electron acceptor, 3-Nitropropionic acid (**3NPA**). This approach was used to gain understanding on the dynamics of bacterial communities in the presence of a hydrogen consuming compound. We incubated rumen fluid *in vitro* across six time points, from 30 minutes to 48 hours after incubation, to compare bacterial composition and fermentation parameters in the rumen with and without 3NPA. The total gas, pH, volatile fatty acids and 16S rRNA (V3-V4 regions) were analyzed. Statistical analysis to observe differences between control and treatment samples were analyzed using RStudio (v.4.3.2), with a *p*-value cutoff of 0.05 across the study. To identify the enhanced taxonomic features between groups for the different time points, LEfSe was used at the cutoff LDA score of 2, and employed the non-parametric Kruskal-Wallis test. The fermentation data confirmed the potency of 3NPA as a methane reducing agent. At 30 minutes to 12 hours, we did not detect any methane production in 3NPA treated samples, while at 24 hours to 48 hours the reduction of methane was still significantly high ($p < 0.001$). The total gas production was only reduced at 30 minutes and 6 hours ($p < 0.05$), which was to be expected due to the selection pressure by microorganisms during the degradation of 3NPA. The volatile fatty acids were positively affected as the total amount significantly increased at 48 hours ($p < 0.01$). Microbiome analysis confirmed that the communities associated with hydrogen consumption were differentially expressed throughout the experiment. These included *Streptococcus*, *Treponema*, *Selenomonas*, *Ruminococcus*, and *Blautia*, along with the family *Lachnospiraceae*. Starting at 12 hours, when the gas production for 3NPA and control were at par, the phylum *Proteobacteria* was significantly enriched, particularly the genus *Sutterella*, in the 3NPA treatment. Interestingly, the putative propionate producers *Succinivibrionaceae* and *Prevotella* were consistently reduced throughout the study. These results suggested that the enrichment of hydrogen metabolizing bacterial communities could have the ability to maintain fermentation while reducing methane. It has been established that alternative hydrogen consuming pathways are effective to compete with methanogens for hydrogen. Our study revealed that 3NPA is an electron acceptor which has the potential to enhance hydrogen consuming bacterial communities, consequently, successfully competing with methanogens for hydrogen and reducing methane production.

317 Improving the identification of rumen bacteria: development and evaluation of a custom MALDI-TOF MS database

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In recent years, efforts to expand pure ruminal culture collections have been made with the intention of improving the understanding of ruminal fermenting microbes, particularly in light of the largely underexplored microbial diversity. This understanding is essential for advancing the development of Direct-Fed Microorganisms (**DFMs**), which hold promise for improving livestock productivity and mitigating environmental impact. Initial identification methods are usual laborious and costly process. In contrast, Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (**MALDI-TOF MS**) has been widely used in clinical applications for the rapid and accurate identification of pathogenic species providing an alternative to traditional methods. By examining the distinctive protein profiles of microorganisms, MALDI-TOF MS allows for precise identification. However, its accuracy is limited to recognising microorganisms that are already included in its database, including closely related species or strains. This study presents the development and evaluation of a customised MALDI-TOF MS database of rumen isolates, aiming to improve the identification of novel bacterial isolates and optimising the expansion of the ruminal biobank of pure cultures. Currently, the rumen-specific MALDI-TOF MS database includes 102 bacterial pure cultures derived from dilution to extinction and direct plating methods, with identification based on partial 16S rRNA gene sequencing. Pure isolate cultures were cultivated on Hobson's M2 agar under anaerobic conditions at 39°C for 48 hours and a colony from the plate was analysed using the Autobio AutoMS1600 MALDI-TOF MS system. The manufacturer library construction method was followed to deposit the isolates into a customised database. Once an isolate was incorporate into the database, colonies from the same plate were picked and processed following the manufacturer's extended method for initial validation. The spectra generated were processed using both the original AutoBio database and the rumen customised database. If the closely related species were already found in the AutoBio database, the reliability scores were compared to evaluate whether the custom database improved the ability of MALDI-TOF MS system to reliably and accurately identify bacteria derived from the rumen. The efficacy of the database was then evaluated using 73 freshly isolates ruminal bacteria. Accurate identification of rumen bacterial species increased with the creation of the rumen database: from 50% to 84%. When comparing the reliability scores of species already in the database, the reliability improved from an average of 9.011 to 9.632 out of a possible score of 10. Further testing with the 73 pure culture samples showed a 65% increase identification efficiency compared to the inbuilt. In conclusion, the customized rumen-specific database for MALDI - TOF MS is valuable for expanding culture collections by reducing the number of novel isolates that require identification through sequencing.

283 Effect of extruded linseed on methane emissions and rumen microbiota in two different diets

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Extruded linseed, rich in polyunsaturated fatty acids, has already demonstrated significant potential for methane mitigation. However, previous research suggests that the effectiveness of extruded linseed may be influenced by interactions with the diet composition. This study aimed to assess the impact of extruded linseed on production parameters, methane emissions and ruminal microbiota when supplemented into two different diets. A 2X2 cross-over experiment was conducted using 39 lactating Holstein dairy cows (155 ± 50 days in milk, 32.1 ± 5.4 kg milk day/day, 688 ± 72 kg bodyweight). Four dietary treatments were included: 1) maize silage-based diet (maize silage:grass silage of 75:25 on dry matter base), 2) maize silage diet supplemented with extruded linseed (400g crude fat per day), 3) grass silage-based diet (maize silage:grass silage of 25:75 on dry matter base), and 4) grass silage diet supplemented with extruded linseed (400g crude fat per day). Balanced concentrates were replaced with extruded linseed, ensuring that the diets remained iso-energetic. The experiment consisted of three treatment periods of 35 days, as such each animal received three different treatments. Data on dry matter intake and milk production were collected daily. Milk samples for analysis of milk composition were taken in the final week of each treatment period. Methane emissions were measured using a GreenFeed system. Rumen fluid was sampled via oral tubing on day 27 or 28. Metataxonomic analysis was performed on rumen samples, targeting four domains: bacteria, archaea, protozoa, and fungi. Outcome variables were analyzed using a linear mixed-effects model, with diet, treatment, their interaction, period and days in milk as fixed effects, and cow as a random effect. Extruded linseed reduced dry matter intake (maize silage diet: 24.2 to 23.7 kg; grass silage diet: 23.9 to 23.0 kg, $P < 0.05$) but increased milk yield and fat-and-protein corrected milk yield (maize silage: 1.0 kg and 0.9kg; grass silage: 1.2 kg and 1.5 kg; $P < 0.01$). Milk composition was altered by extruded linseed, with decreasing ($P < 0.05$) protein (0.11% points) and milk urea nitrogen (7 mg/L) and increasing ($P < 0.01$) lactose (0.05% points) in both diets. Milk fat content reduced only in the grass silage diet (-0.15 % points, diet × treatment $P = 0.064$). Methane production (g/day) and intensity (g/kg milk, g/kg fat-and-protein corrected milk) decreased ($P < 0.01$) by 5% and 7% across both diets because of the addition of extruded linseed. Alpha and beta diversity of bacteria and fungi were significantly influenced by diet ($P < 0.05$), with greater diversity observed in the grass silage-based diets. For Archaea, both alpha and beta diversity were affected by diet and treatment ($P < 0.01$), with extruded linseed exerting a more pronounced impact resulting in a lower alpha diversity (Shannon index and weighted UniFrac) in the maize silage-based diet. The alpha and beta diversity of protozoa were not affected by diet or treatment. To conclude, extruded linseed supplementation reduced methane production and intensity without affecting production parameters, with preliminary microbiota results suggesting mainly a diet-dependent effect. Further analysis of indicator species and functionality is ongoing to better understand these microbial responses.

100 Exploring microbial-enhanced selection to lower methane emissions from dairy cows

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Worldwide, dairy farmers are seeking tools to reduce methane emissions. Genomic selection is a promising strategy. Enteric methane is however not directly synthesized by the dairy cow, but by its rumen microbiome, parts of which are influenced by the cow's genetics. This relationship suggests that genetic selection could be applied on both, the direct host genotype-phenotype axis and the indirect host genotype-microbiome-phenotype axis. Including microbial data in genetic selection models may therefore improve future selection strategies for mitigating methane. Our study aimed to (i) assess if microbial-enhanced selection could improve genomic selection for lower methane, and (ii) identify microbial patterns which might be targeted by selection for lower methane. Our dataset included 642 Holstein cows from 10 Dutch dairy farms with daily methane concentrations (ppm via sniffers), pedigree, genotypes, and milk records. Rumen fluid was collected once per cow. methane phenotypes were established by averaging all records per cow within a time frame of ± 50 days from the rumen fluid sampling date. Rumen microbial data was derived from long-read metagenomic sequencing and analysed using the SqueezeMeta software. Genetic parameters were estimated using ASReml version 4, with three models modelling:

1. Host genomic data only,
2. Microbial data only,
3. Combined genomic and microbial data.

Log-likelihood ratio tests were used to compare the models. Heritabilities of microbial taxa and gene abundances were estimated, and heritable taxa and gene subsets were further associated to methane concentration. Mean methane concentrations per cow and day were on average 436.2 ppm, ranging from 87.3 to 863.8 ppm ($Q1 = 310.5$, $Q3 = 539.5$). Results of the models were for model 1: heritability (h^2) = 0.21 (standard error (**SE**) = 0.08), for model 2: microbiability (m^2) = 0.11 (SE = 0.07), and for model 3: $h^2 = 0.20$ (SE = 0.08), $m^2 = 0.10$ (SE = 0.07). The combined model fitted the data significantly better than model 1 (p-value < 0.024) and model 2 (p-value < 0.01). Analyses of the microbial dataset indicated a core rumen microbiome constituting 577 genera, 152 of which were found to be heritable. *Parasporobacterium*, unclassified *Peptococcaceae*, and *Pantoea* were of major interest due to their high heritability and association to methane concentration. Out of 18,804 mapped genes, 132 gene abundances were associated with methane related pathways. Eleven of these genes were of major interest due to their significant heritabilities (significance defined as: $h^2 = h^2 - (SE \cdot 1.96) > 0$) and association to methane concentration. These genes were annotated within transferase, hydrolase, and oxidoreductase pathways. Our analyses showed that including microbial data into a genomic model improves the phenotypic prediction of enteric methane concentrations. Moreover, our analyses unraveled potential microbial key players which might be targeted by genetic selection to reduce methane emission in dairy cows.

313 Supplementation with monomeric phenolic compounds modulates the rumen bacterial community under inhibited methanogenesis *in vitro*

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Inhibition of rumen methanogenesis results in the accumulation of dihydrogen, which is not used by rumen microbes to produce fermentation products of nutritional value to the host ruminant. We previously reported that monomeric phenolic compounds significantly decreased dihydrogen accumulation and increased total VFA production *in vitro*. Moreover, this effect was concomitant with an increase in acetate concentration, suggesting that these compounds can stimulate the growth of microbes whose metabolism involves the fermentation of these compounds to acetate. Here, we used a metataxonomic approach to gain insight into the possible changes in the rumen microbiota induced by phloroglucinol (**PHL**) and pyrogallol (**PYR**) with and without the addition of methanogenesis inhibitors, specifically 2-bromoethanesulfonate (**BES**), *Asparagopsis taxiformis* (**AT**). We compared the response to this supplementation using rumen fluid from two ruminant species, dairy cows (n=8) and goats (n=4). For each animal species, we performed sequential *in vitro* incubations for up to seven days and targeted both the bacterial and archaeal communities with a universal 16S RNA gene primer. There were four treatments in the cow experiment: CTL, (control), BES (3 μ M), PHL (36 mM), and BES+PHL (3 μ M BES + 36 mM PHL). There were six treatments in the goat experiment: CTL (control), AT (2% inclusion in DM), AT+PHL1 (2% AT + 6 mM PHL), AT+PHL2 (2% AT + 36 mM PHL); AT+PYR1 (2% AT + 6 mM PYR), and AT+PYR2 (2% AT+ 36 mM PYR). In both experiments, the number and diversity of methanogens were greatly reduced at the end of the incubation and were not included in the diversity analysis. For bacteria, the antimethanogenic treatments did not affect neither alpha or beta diversity compared to controls. Conversely, in both cows and goats alpha diversity indices exhibited a significant decline in response to the supplementation with phenolic compounds. A PCoA analysis demonstrated convergence of microbial communities with treatments containing 36 mM phenolic compounds which was confirmed by Adonis (R^2 0.72 $p < 0.001$). Cow and goat samples displayed a notable grouping pattern also confirmed by a post-hoc testing. For bacteria, most taxa with increased abundance (including Lactobacillaceae, Bifidobacteriaceae and Lachnospiraceae) were described as able to utilize phenolic compounds, whereas the abundance of cellulolytic bacteria (including Fibrobacteraceae, Ruminococcaceae and Succinivibrionaceae) decreased with this supplementation. Although the structure of the rumen bacterial community differed between cows and goats, about half of the bacterial clades whose abundance was affected by 36 mM PHL in the cow experiment were also affected by 36 mM PHL and PYR in the goat experiment. These results suggest that dietary supplementation with phenolic compounds could significantly modify the rumen bacterial community towards more efficient rumen dihydrogen metabolism and ultimately as a potential methane mitigation strategy. However, future studies should also consider a possible negative effect on fibre degradation.

249 Comparison between microbiome of ruminal fluid and faeces from dairy cows in two grazing-based systems

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Research on cattle gut microbiome has focused on the complex and dynamic ruminal microbes and how they are affected by feeding, use of additives, plant secondary compounds, among other practices. However, few studies have examined the dynamics of the microbiome structure at the distal end of the gastrointestinal tract (**GIT**), represented by faecal samples. This study aimed to evaluate the ruminal and faecal microbiome diversity of 10 Holstein cows randomly assigned to graze on two treatments: 1) *Cenchrus clandestinus* grass as the traditional system (**TS**) and 2) silvopastoral arrangement (**SPS**), composed of *Cenchrus clandestinus* grass plus *Sambucus peruviana* shrubs in Mosquera (Cundinamarca, Colombia at 2.550 m.a.l.s). Rumen and faecal metagenome sequences were obtained using 16S rRNA gene amplicon sequencing. Reads were processed using the Illumina BaseSpace 16S rRNA application module (2013 Illumina-curated version) with the Greengenes taxonomic database, alongside the Ribosomal Database Project (**RDP**). Experimental procedures for this study were approved by the AGROSAVIA Honorary Committee on Ethics in the Use of Animals. To evaluate the microbiota composition at genus level sequences greater than 2% of the total reported across all samples were compared between treatments and sample types. Genus with lower abundance were grouped into the category "others." Shannon index was calculated considering all genus found in both samples type from TS and SPS to evaluate the richness and diversity. The effect of the treatment and the sample type on the Shannon richness index and the microbiota composition was evaluated using a completely random design with a 2x2 factorial arrangement. The Tukey multiple test comparison was used when significance differences ($P < 0.05$) were met. As results, a total of 1772 genus were found in the faecal and rumen fluid samples. Although the Shannon index demonstrated a high diversity (>3) in the microbiota across both treatments and type of samples, no significant differences were observed between TS and SPS with values of 3.76 and 3.68, respectively. However, within sample types significant differences ($P < 0.002$) were observed, with values of 3.98 and 3.47 for faecal and ruminal fluid samples, respectively. The dominant genus across treatments and sample types was *Prevotella*, representing the highest proportion of total sequences (11.6%). Other key microbial genus across samples were *Bacteroides*, *Lysinibacillus*, *Serpens*, *Stenotrophomonas*, *Solibacillus*, *Bacillus*, *Saccharofermentans* and *Acinetobacter* represented by 8%, 6.8%, 4.1%, 3.4%, 2%, 1.9%, 1.8%, and 1.6%, respectively. No significant differences ($P > 0.05$) were found in genus with a presence greater than 2% between TS and SPS. Faecal and rumen fluid samples were significantly different, with 4386.6, 10425, 8905.2, and 2338 sequences of *Bacteroides*, *Lysinibacillus*, *Serpens*, and *Solibacillus* in faecal samples, and *Prevotella* in rumen fluid with 15003 sequences in contrast with 361 sequences found faecal samples. Although this study presents results from a small experimental animal size, it highlights the potential of large intestine microbiome characterization to improve understanding of synergies and networks across the cattle GIT. Increasing the sample size in future research could help validate findings and identify factors modulating microbial population structure through different forages or treatments in larger livestock populations.

45 Effects of nitrate, protein supply, and genetic yield index on dairy cow rumen microbiota

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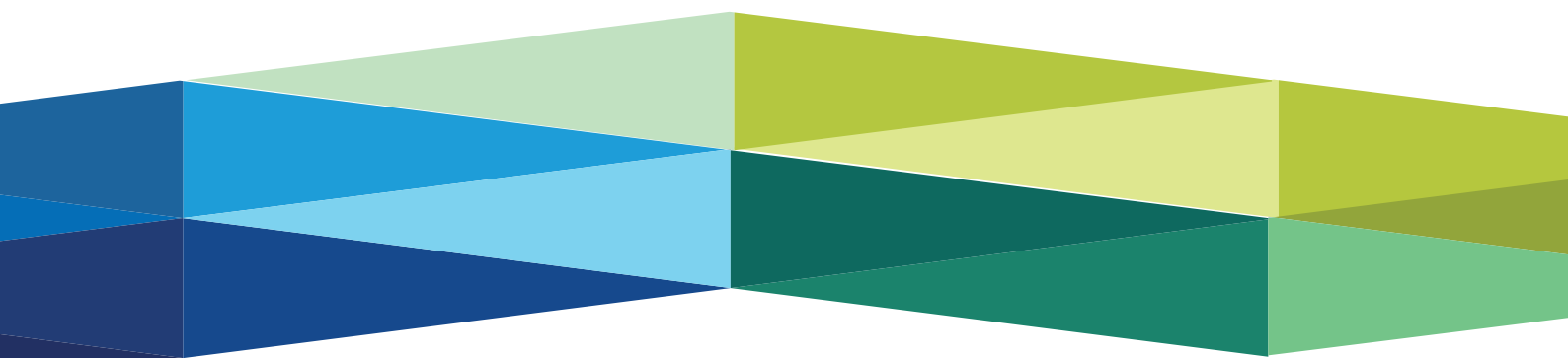
Nitrate not only exhibits significant potential for methane mitigation but also serves as a non-protein nitrogen source, similar to urea. This study aimed to investigate the interaction between non-protein nitrogen sources, dietary rumen degradable protein/rumen undegradable protein ratio, and production level (genetic yield index, 78-152) on the rumen microbial community in dairy cows. Twenty-four multiparous Danish Holstein dairy cows were assigned to a 6 × 4 incomplete Latin square design. Cows were blocked into 4 groups based on days in milk and milk yield. Six experimental diets were formulated in a 2 × 3 factorial arrangement: 2 non-protein nitrogen sources (nitrate or urea) and 3 rumen degradable protein/rumen undegradable protein ratios (low, medium, and high; 2.50, 3.14, 3.91). Feed intake, milk yield, gas emissions, and nutrient digestibilities were determined. Rumen fluid was collected for DNA extraction and V3-V4 region of the 16S rRNA gene was sequenced. Statistical analyses were conducted in R (version 4.4.1) using a linear mixed model that included dietary rumen degradable protein/rumen undegradable protein ratio, non-protein nitrogen source, parity, period, and genetic yield index (as a continuous variable) as fixed factors, and cow as random factor. All two-way and higher-order interactions among fixed factors were also included in the model. Neither rumen degradable protein/rumen undegradable protein ratio nor genetic yield index showed a significant effect on the Shannon index or the Evenness of the bacterial community. However, nitrate supplementation resulted in a lower Shannon index and Evenness compared to cows receiving urea ($P < 0.05$). Neither nitrate supplementation, rumen degradable protein/rumen undegradable protein ratio, nor genetic yield index affected the β diversity based on unweighted UniFrac distance ($P > 0.05$). At the phylum level, the relative abundance of Patescibacteria increased linearly with the increasing dietary rumen degradable protein/rumen undegradable protein ratio ($P = 0.03$). In comparison to urea supplementation, nitrate supplementation led to a higher relative abundance of Actinobacteriota ($P = 0.05$) and a lower relative abundance of Patescibacteria ($P < 0.01$). The genetic yield index had no effect on the bacteria composition at the phylum level ($P > 0.05$). At the genus level, the relative abundance of *Prevotellaceae_UCG.003* increased linearly ($P = 0.02$), while the relative abundance of *Clostridia_UCG.014* decreased as the dietary rumen degradable protein/rumen undegradable protein ratio increased ($P = 0.05$). Compared to urea supplementation, nitrate supplementation resulted in a lower relative abundance of Ruminococcus ($P < 0.01$) and *Clostridia_UCG.014* ($P < 0.02$), but a higher relative abundance of *Prevotellaceae_UCG.003* ($P < 0.01$). In conclusion, nitrate supplementation lowered α diversity and influenced microbial composition, whereas the dietary rumen degradable protein/rumen undegradable protein ratio and the genetic yield index showed no significant effects on ruminal microbial community, except on the relative abundance of a few bacteria genera.



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Theme 4: Manure management



243 Reducing ammonia emissions from dairy slurry storage by using *Himanthalia elongata* as a dietary additive

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Livestock production is a major source of ammonia emissions, primarily arising from slurry management. These emissions contribute to environmental challenges such as acidification, eutrophication, and the formation of particulate matter, posing significant risks to ecosystems and human health. To mitigate these impacts, various strategies have been explored, with dietary additives emerging as a promising approach. Bioactive compounds in plants, such as condensed tannins, have demonstrated their ability to bind with proteins in the rumen, reduce protein degradation, shift nitrogen from urine to faeces and ultimately lowering ammonia emissions from slurry. Similarly, phlorotannins derived from brown seaweed have shown comparable mechanisms, although their effect on ammonia emissions from dairy cow slurry during storage has not been explored. The aim of this study was to investigate, for the first time, the effect of supplementing dairy cow diets with *Himanthalia elongata*, both as whole seaweed and seaweed extract, on ammonia emissions from slurry during storage.

Nine late-lactation multiparous Holstein-Friesian cows, balanced for parity, body weight, and average milk yield, were randomly assigned to one of three dietary treatments for 22 days. All diets contained 40% concentrates on a dry matter basis: control (60% grass silage), whole seaweed (56% grass silage and 4% dried *Himanthalia elongata*), and seaweed extract (56% grass silage and 4% *Himanthalia elongata* hydrolysate). Urine and faeces samples were collected separately from each cow, with urine from three cows within each treatment group pooled together and homogenized, following the same procedure for faeces. The urine and faeces from each treatment were then combined in a 2:3 urine-to-faeces ratio to form 20 litres of slurry. Slurry samples were prepared in triplicate for each treatment and stored in 30-litre containers. Ammonia emissions were measured using the LumaSense Innova 1512 photoacoustic gas analyser over six weeks with the static chamber method. Differences in emissions across treatments were evaluated using one-way ANOVA and post-hoc Tukey tests ($P < 0.05$).

Over the six weeks of slurry storage, the cumulative ammonia flux was 733.70 ± 66.46 mg N/m² for the whole seaweed treatment and 996.71 ± 26.94 mg N/m² for the seaweed extract treatment, compared to 1379.64 ± 92.76 mg N/m² for the control treatment. The whole seaweed diet was more effective than the seaweed extract diet in reducing ammonia emissions, with reductions of $43.92 \pm 4.82\%$ and $29.93 \pm 1.95\%$, respectively. Additionally, analysis of phlorotannins in the slurry revealed that cows fed the whole seaweed diet had significantly higher phlorotannin levels (6.66 ± 1.40 µg PGE/mL) compared to those fed the seaweed extract diet (0.66 ± 0.76 µg PGE/mL) which contributed to more significant reduction in ammonia emissions. These findings provide the first evidence of the potential of brown seaweed, *Himanthalia elongata*, as an effective and sustainable dietary intervention to reduce ammonia emissions during slurry storage in dairy farming.

199 GLEA-Stat Manure: The first global database of manure management systems

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Manure is vital for sustainable agriculture, serving as a cost-efficient fertilizer rich in nutrients and organic matter that enhances soil health and crop productivity. However, when mismanaged, manure becomes a source of environmental concerns, contributing to water pollution and significant greenhouse gas (GHG) emissions, particularly nitrous oxide (N₂O) and methane (CH₄). Effective manure management is therefore essential to maximize its benefits while minimizing its environmental footprint. The most recent global assessment of GHG emissions from livestock agrifood systems, conducted using the Global Livestock Environmental Assessment Model (GLEAM), revealed that manure management accounts for nearly 13% of total livestock-related GHG emissions. These emissions are largely influenced by the type of manure management systems (MMS) in use, highlighting the need for detailed and accurate data to identify effective mitigation strategies. A consistent, spatially explicit global data set with up-to-date information on MMS for all species is currently lacking, making global scale assessments of emissions from manure and their mitigation potential difficult. Using a variety of data sources such as peer-reviewed studies, scientific databases, technical reports, and country statistics, we have compiled a global data set of MMS with granular information on the proportions of manure managed across different systems, classified by country, species, and livestock production systems. These data are aligned with the manure management categories defined by the Intergovernmental Panel on Climate Change (IPCC). This new data set (GLEA-Stat Manure) will enable more accurate global calculations of GHG emissions. Importantly, it will support the identification of critical hotspots for mitigation interventions, helping to reduce emissions and promote the circular use of manure as a valuable resource in agriculture. We will present an overview of the approach and the data sets as well as initial application to estimate emissions, mitigation potential, and nutrient flows.

102 Evaluation of manure emission management strategies and greenhouse gas management in Tabora region, Tanzania

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In Tabora Region, Tanzania, many locals rely on livestock for their livelihoods, raising cattle, goats, and poultry. However, traditional manure management methods, driven by limited resources, lead to high emissions of methane and nitrous oxide, which contribute to global warming. These greenhouse gases (**GHGs**) impact livestock farming, and understanding their sources and ways to reduce emissions in this context is essential. This study aims to assess manure management practices in Tabora and their resulting GHG emissions.

The study involved 40 farms across 40 villages in Tabora, including smallholder cattle, poultry, and mixed farms. It examined common manure management practices, such as slurry storage in pits, composting, and direct application to soils. GHG emissions were measured by collecting samples from manure storage sites (piles and slurry) using static chambers, focusing on methane and nitrous oxide. Emissions were quantified through gas chromatography, and CO₂ equivalent emissions were calculated based on IPCC guidelines for livestock systems.

Data were analyzed using ANOVA to compare GHG emissions across different manure management systems. Regression analysis examined the relationship between fertilizer management practices, farm characteristics (e.g., farm size, livestock type), and GHG emissions, with statistical significance set at the 95% confidence level. The study found significant variation in GHG emissions based on manure management methods. Farms with open slurry storage had the highest methane emissions, averaging 30 kg CH₄ per hectare per year, accounting for about 60% of total GHG emissions from manure. Composting reduced methane emissions by 40%, while farms directly applying manure had minimal methane emissions. Nitrous oxide emissions were higher in systems using organic fertilizers like compost and slurry storage. Additionally, regional factors such as rainfall and temperature were key predictors of GHG emissions. Manure management practices in Tabora contribute significantly to GHG emissions, particularly methane from open slurry storage. Alternatives like composting or direct manure application could reduce emissions. The study emphasizes the need for region-specific recommendations and support to help farmers adopt more sustainable practices. Policy interventions, including financial incentives and education, are crucial for reducing GHG emissions and enhancing the environmental sustainability of livestock farming. Future research should focus on the economic feasibility and scalability of these mitigation strategies in Tabora.

225 An overview of animal manure management in Nigeria

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In Nigeria, where agriculture plays a major role in the national economy, managing animal waste is essential to sustainable livestock production. However, poor manure management techniques can have negative effects on the environment, human health, and the economy. An overview of animal manure management in Nigeria is given in this paper, together with information on present procedures, difficulties, and areas for development. Poultry, cattle, and pigs are the main sources of the 12.6 million tonnes of manure produced annually in Nigeria. An estimated 70% of farmers currently use open dumping or burning as their primary methods of managing manure. This has led to greenhouse gas emissions, water pollution, and environmental deterioration. Inadequate infrastructure, restricted financial access, and a dearth of legal frameworks are ongoing obstacles to efficient manure management in Nigeria. Composting, biogas production, and the implementation of integrated manure management systems present opportunities for improvement. These would maximize nutrient recycling, improve sustainable agricultural productivity, and lessen the negative consequences of greenhouse gas (**GHG**) emissions. Improving these methods would benefit the economy by recovering resources and producing energy, in addition to helping to maintain a sustainable environment. In order to support sustainable livestock production and environmental stewardship, this systematic review emphasizes the necessity of a comprehensive strategy to animal manure management in Nigeria that includes technological, socioeconomic, and legislative interventions. The data supporting these assertions were derived from a survey of top scientific literature covering the years, 2011-2022. In addition, primary data was sourced over a 9-month period across Nigeria (December 2022-August 2023) on methods of disposing poultry waste.

415 Evaluating acidification rates on methanogen populations in stored liquid swine manure under realistic Canadian temperatures

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Livestock manure is a significant source of methane (CH_4), a potent greenhouse gas with substantial climate impacts. In Canada, agriculture contributes 8.1% of national GHG emissions, with swine manure alone emitting 1,700 kiloton CO_2 eq annually. Most emissions occur during warmer months, increasing the carbon footprint of Canadian pork production. Methane reduction on farms remains voluntary, highlighting the need for cost-effective solutions. Our study focuses on Manitoba, a leading swine-producing prairie province in Canada. This study evaluates the low-dose sulphuric acid's effectiveness in mitigating CH_4 emissions from swine manure, and the microbial processes driving these changes. Anaerobic incubation bottles containing liquid swine manure (500 mL for methane measurements and 1 L for microbial sampling) were treated with one of four sulphuric acid (H_2SO_4 , 95–98%) application rates: R1 (0.7 mL/L), R2 (1.4 mL/L), R3 (2.1 mL/L), and R4 (2.8 mL/L). A control treatment (no H_2SO_4) was also included, for baseline comparison across treatments. Each treatment was tested at four temperature levels: 17, 20, 23, and 37°C, with three replicates per treatment-temperature combination. Bottles were incubated for 184 days, and methane emissions were quantified using gas chromatography when headspace pressure exceeded 10 kPa. Manure was sampled biweekly, preserved, and stored at -80°C for microbial analysis. DNA and RNA were co-extracted and analysed for active methanogen (*mcrA*) using quantitative-PCR and shifts in the bacterial and archaeal communities through high-throughput sequencing. Methane emissions showed strong temperature dependency. At 37°C, emissions from the control increased rapidly, plateauing by day 87 with cumulative emissions of 4456 ± 413 mL. In contrast, at 23°C, emissions took over twice as long to reach comparable levels, highlighting the slower rate of methane production at lower temperature. At 20°C, methane production started gradually after 100 days, while at 17°C, emissions remained minimal. Quantification of *mcrA* gene expression indicated that methanogenic activity was significantly suppressed under a colder temperature. The addition of H_2SO_4 delayed methane production across all temperatures compared to the control. At higher rates (R3 and R4), methane was completely suppressed at 17°C and 20°C, while at 23°C, suppression lasted for up to 130 days. At R2, suppression was less pronounced, with emissions resuming earlier, particularly at 23°C. Higher rates resulted in minimal emissions even after 184 days of storage. For lower rates, the effect of H_2SO_4 diminished over time, especially at higher temperature. The delayed onset of methane emissions at R2 correlated with a gradual recovery of methanogen populations after day 87, particularly at 20°C and 23°C. These observations highlight the temperature- and dose-dependent effects of H_2SO_4 on methanogenic activity and methane suppression over time. Samples are currently being analysed by high throughput sequencing to identify shifts in methanogen diversity linked to methane emissions across treatments, and treatment interaction results will be shared at the conference. This study advances understanding of methanogenesis drivers in manure systems and highlights how sulphuric acid effectively mitigates methane emissions, particularly under low temperature storage conditions.

101 Impact of antibiotics on greenhouse gas and ammonia emission from solid manure storage in Kenya

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The increasing use of antibiotics in livestock farming raises concerns about environmental impacts. This study examined the effects of three antibiotics commonly used in Kenya – Tylosin, Enrofloxacin, and Oxytetracycline – on manure greenhouse gas (**GHG**), ammonia (**NH₃**) emissions, and fertilizer quality. To determine appropriate concentrations for the manure spiking, a review of recommended dosages of the antibiotics from manufacturers' data sheets was conducted based on the live weight of local *Boran* cattle weighing 200 kg. Average excretion rates of 80% for Oxytetracycline, 40% for Enrofloxacin, and 70% for Tylosin were used to estimate antibiotic residue levels in manure, assuming an average daily manure output of 10 kg Fresh Weight (**FW**) per cow. These values informed the dosage applied to 10 kg FW of manure in this study. High and low antibiotic concentrations for each drug were established by assuming the antibiotics would be excreted over a one-day (high concentration) and seven-day (low concentration) period. Aliquots of fresh cattle manure (10 kg) were spiked with 100 mg kg⁻¹ (low), 700 mg kg⁻¹ (high) Tylosin, Enrofloxacin 29 mg kg⁻¹ (low), 200 mg kg⁻¹ (high), and Oxytetracycline 229 mg kg⁻¹ (low) and 1600 mg kg⁻¹ (high) dry weight manure assuming a manure moisture content of 20 %, plus a control without antibiotics, and incubated in open buckets for 110 days to simulate manure heaping. Emissions of methane (**CH₄**), nitrous oxide (**N₂O**), and **NH₃** were measured via a static chamber using a Picarro laser spectrometer. Results showed that Tylosin (high and low) treatments and high-dose Enrofloxacin significantly increased **N₂O** emissions, surpassing IPCC default values of 1% manure-N.

All treatments increased **CH₄** emissions compared to the manure-only control and raised **CH₄** emissions factors (above the IPCC default value of 4.44 g **CH₄** kg⁻¹ VS) except the control (2.22 ± 0.5 **CH₄** kg⁻¹ VS). The **NH₃** emission factors for manure with antibiotics were the same or less than the Environmental Protection Agency factor of 20% manure-N. Overall, the antibiotics increased the global warming potential from solid manure management by 218% compared to the control.

We concluded that antibiotics increase manure greenhouse gas emissions. The increased **CH₄** emissions could be due to antibiotics affecting methanotrophs (methane-consuming bacteria) by reducing their abundance, while the increased **NH₃** and **N₂O** could be due to antibiotics disrupting the electron transfer system in microbial metabolic pathways by inhibiting enzymes related to ammonification and denitrification. Key enzymes involved in this process include urease, nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, with corresponding encoding genes *ureC*, *narG*, *nirK/nirS*, *norB*, and *nosZ*. While **NH₃** and **N₂O** emissions led to higher N loss, the manure fertilizer quality remained unaffected. Besides health concerns (antimicrobial resistance), our study of antibiotic environmental impact calls for a One Health approach for sustainable antibiotic use and better manure management in livestock.

360 Evaluation of novel treatments to reduce ammonia and greenhouse gas emissions from stored cattle slurry

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Cattle slurry contains valuable plant nutrients and improves soil fertility for crop growth after field application. Slurry is stored in slurry tanks for a few months prior to the field application due to the field being occupied by the crops or regulatory provisions. During storage, slurry undergoes several physico-chemical transformations which produce environmentally malignant gases such as ammonia (NH_3) and greenhouse gases (GHGs). Losses of nutrient in the form of NH_3 and GHGs also decrease the nutrient values of the slurry. Therefore, novel treatments and effective strategies are essential to reduce the emissions during storage. In this study, we quantified NH_3 and GHGs emissions and potential emission reduction efficiencies of floating covers, commercial additives and aeration systems comparing them in a pilot-scale storage facility under realistic storage period.

The experiment was conducted for 134-d under pilot-scale slurry storage facility of AFBI-Hillsborough research farm. The storage facility has 18 underground concrete storage tanks (1m^3) with a flow-through system, thus the tank headspace is continuously flushed with fresh air. We stored 700 L slurry in each tank and amended with treatments. The treatments were: dairy cattle slurry as control, light expanded clay aggregates (LECA; 10cm on surface), biochar made from the solid fraction of digestates (2cm on surface), slurry aeration at low frequency (3 min every 12 h) and slurry aeration at high frequency (3 min every 6 h) and a commercial additive (GasAbate: hydrogen peroxide-based treatment). These treatments were randomly assigned to the tanks. During the experiment, NH_3 and GHGs concentrations were continuously monitored from each tank sequentially using a multipoint sampler (Innova 1409) and photoacoustic gas analyzer (Innova 1512). Three gas samples were collected from a tank before switching to the next tank during measurement and the first data point was discarded during analysis to remove potential carry over effect. Ambient air samples were taken from both ends of the storage facility to correct the concentrations. Airflow and air temperature were recorded every minute that was used to calculate the fluxes. Statistics of the data were performed using ANOVA tests under R-programming language.

Treatment effects for NH_3 emissions were in the order of LECA < biochar < GasAbate < high frequency aeration < low frequency aeration < cattle slurry. LECA ($56.26 \pm 7.84 \text{ g NH}_3\text{-N/m}^2$) and biochar ($201.09 \pm 24.91 \text{ g NH}_3\text{-N/m}^2$) reduced ($p < 0.05$) NH_3 emissions by 83% and 40%, respectively, compared to slurry ($336.62 \pm 18.18 \text{ g NH}_3\text{-N/m}^2$). Use of biochar (215%) and LECA (57%) as floating covers significantly ($p < 0.05$) increased the N_2O emissions compared to untreated cattle slurry. For CH_4 emissions reduction, GasAbate was the most effective treatment followed by the high aeration system and LECA.

This study demonstrates that mitigation of NH_3 and GHGs emissions is a complex process as a treatment may effectively reduce NH_3 emissions, but may increase GHGs emissions or vice versa. Thus, both GHGs and NH_3 emissions need to be monitored to effectively inform policy development. Considering both NH_3 and GHG emissions data, GasAbate, LECA and high frequency aeration can be an environmentally friendly option among the compared treatments.

64 Assessment of integrated dairy farming with vermicomposting technology in Gondar City, Amhara region, Ethiopia

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The main objective of this study was to assess integrated dairy farming with vermi-composting technology in Gondar City, Northwest, Ethiopia. The study had both a qualitative and quantitative research approach. Data were gathered through structured and semi-structured questionnaires, group discussions, direct observations, and interviews with key informants. Seventy-three randomly selected dairy farm operators were surveyed face-to-face. Additionally, three focus group discussions, each with 6-10 participants (totaling 24 individuals), and interviews with five knowledgeable key informants on dairy farming were conducted. Focus group participants included local leaders, agricultural extension workers, exemplary dairy farmers and businesses, and respected community members (elders, women, and men) with insights into the area's past and present dairy practices to ensure reliable information. All survey and interview data, including geographical location via a geo-tagging system, were collected using Kobo Toolbox software. The data were then managed using the same software, exported to Microsoft Excel, and analyzed using SPSS and Excel. The validity and reliability of the data was confirmed with a Cronbach's alpha coefficient of 0.925.

The selected individual respondents, key informants and focus group participants were found to be a useful entity to the Assessment of Integrated Dairy Farming with Vermi-composting Technology. The socio-demographic profile and physical aspects of the dairy enterprises were determined in terms of sex, age, educational attainment, knowledge of dairy farming, years of operation and experience, perception about economical benefits of vermi-compost production. In this study (85%) of the respondents were males, found to be under their productive ages and (75.3%) of them had knowledge's about dairy farming. Whereas, 97.3 % of the respondents had poor knowledge and none of them were received institutional support like training about vermi-composting production from any pertinent offices. According to the respondents (74%) had very low, low (23.3%) and medium extension service (2.7%) about vermi-compost production. Most (80.8%) of the respondents had moderate perception while 18.3% and 15% had less and high perception of the beneficial effects and limitations of vermi-compost, respectively.

Findings confirmed that majority of the respondents had moderate perception both for profitability advantages, economical and climate impacts of vermin-composting. However, the challenges of vermi-compost production had negatively impacted the views of respondents to adopt it.

The study concluded that dairy enterprises in the area require significant capacity building support from relevant local institutions to effectively develop and implement vermi-composting technology.

371 Enhancing indoor air quality and health among biogas digester users: a case study in Ethiopia

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Household biodigesters, which convert livestock manure into biogas and organic fertilizer, are a sustainable technology adopted in several Sub-Saharan African (**SSA**) countries for clean energy and organic fertilizer production and greenhouse gas mitigation. Biogas use replaces polluting biomass and fossil fuels, which currently expose 1 billion people to indoor air pollution (**IAP**), causing 700,000 premature deaths annually in SSA. Generating evidence on the health effects of climate change mitigation interventions, such as biodigesters, in SSA is essential to drive ambitious, health-protective mitigation actions. This study fills this knowledge gap by measuring active biogas use and its impact on indoor air quality (PM_{2.5} and CO levels), as well as the associated self-reported health benefits, using households in the Gamo and Wolaita zones of southern Ethiopia as a case study.

A total of 421 households (n=227 biogas users and n=194 non-users) were surveyed to evaluate cooking energy patterns, energy sources, kitchen conditions, and health risks associated with IAP. Detailed 24-hour PM_{2.5} and CO concentration measurements were carried out in 41 randomly selected households (n=30 biogas users and n=11 non-users) and among 10 female cooks (5 biogas users and 5 non-users) using low-cost sensors. Additionally, continuous two-week measurements of PM_{2.5} and CO concentrations were conducted in households that used either biogas or firewood exclusively.

The results indicate that exclusive biogas users primarily relied on biogas as their main cooking energy source, while partially switched users supplemented by firewood (98.1%) and charcoal (46.3%), and biogas 41.4% for lighting; when independently analyzed. In contrast, non-biogas users predominantly relied on firewood (100%) as the main cooking energy source, complemented with charcoal (33.5%), agricultural residues (11.9%), and kerosene (54.6%) for lighting. Kitchen conditions also varied significantly between the two groups. Among biogas users, 63% had clean kitchens, with 15% having chimneys and 41.1% having kitchens with windows, and only 25.1% reported black soot, indicating better ventilation and cleaner kitchen conditions. Conversely, non-users had fewer clean kitchens (7.7%), with 44.8% having chimneys, 48.5% having kitchens with windows, and 68.6% reporting black soot, reflecting the demand for ventilation, cleanliness, and layout disparities. Biogas users recorded significantly lower kitchen PM_{2.5} and CO concentrations (53.61 µg/m³ and 3.13 ppm, respectively) compared to non-users (519.26 µg/m³ and 11.5 ppm). Households exclusively relying on biogas for cooking achieved maximum PM_{2.5} and CO levels of 21.60 µg/m³ and 12.66 ppm, respectively on average within WHO indoor air quality standards. In contrast, on average, firewood-dependent households recorded 3042.37 µg/m³ PM_{2.5} and 53.2 ppm CO, far exceeding these limits. Self-reported health outcomes indicated reductions in eye infections, respiratory diseases, and smoke exposure by 62.1%, 61.7%, and 97.8%, respectively, among biogas users. The study concludes that biogas use significantly reduces IAP and associated health risks, with PM_{2.5} emissions reduced by 86.4% and CO by 63.4% compared to firewood kitchens. However, the persistence of energy stacking underscores the need for targeted interventions to promote exclusive biogas use or its combination with other clean fuels to maximise health benefits.

454 Effects of dietary strategies for methane mitigation on greenhouse gas emissions during manure storage

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Dairy farming is a significant source of greenhouse gas (**GHG**) and ammonia (**NH₃**) emissions, posing environmental challenges. While dietary strategies, such as feed additives and modified feeding strategies, have shown promise in reducing enteric methane (**CH₄**) emissions, trade-offs in the form of higher GHG emissions during manure storage are rarely documented. Previous studies have generally been conducted on a smaller scale, with fewer animals, limited storage volumes, and shorter storage durations. Only one study (Uddin et al., 2020; J. Cleaner Prod. 270, 122461) included both storage and field application, as is planned for this study, and their storage phase was conducted at constant temperature in a laboratory environment. This experiment was conducted at pilot-scale under realistic storage conditions to examine how different proportions of maize and grass in dairy cow diets influence CH₄, NH₃, and nitrous oxide (**N₂O**) emissions during manure storage under Danish climatic conditions. The stored manure will later be applied to maize as a fertiliser.

Manure was collected on 12 September 2024 from a four-week feeding trial with 96 cows, half of which received high-maize diets (80:20 maize: grass-clover silage - **MAIZE**) and the other half low-maize diets (20:80 maize:grass-clover silage - **GRASS**). In both cases, silages constituted 60% of dietary dry matter (**DM**), and starch levels were 212 and 150 g kg⁻¹ DM in MAIZE and GRASS diets, respectively. The pilot-scale storage experiment took place in six ventilated (32 m³ m⁻² h⁻¹) storage tanks with a capacity of 6.5 m³, and emissions from c. 3.5 m³ portions of manure were monitored until April 2025. GHG emissions were measured by continuously sampling ventilation air with a peristaltic pump, and collecting time-integrated gas samples in alufoil bags for seven days before analysis. NH₃ emissions were captured using acid traps containing 20 mM phosphoric acid, which were replaced weekly.

During storage, slurry from cows fed the MAIZE diet resulted in significantly higher cumulative CH₄ emissions than from those fed the GRASS diet during the first 2-3 months of storage. Manure NH₃ emissions (an indirect source of N₂O) were 44% higher for the cows fed the MAIZE diet, while N₂O emission levels were similar. The overall GHG balance was dominated by CH₄, which constituted c. 90% of emissions. The absolute and relative contribution of CH₄ to GHG emissions from manure storage increases with storage temperature, and modelling will be used to extrapolate the observed emissions to average annual emissions.

The higher CH₄ emissions of slurry from cows fed the MAIZE diet compared to those fed the GRASS diet contrasts with enteric emissions where the GRASS diet produced 18% more CH₄ than the MAIZE diet (C.F. Børsting, this conference). It suggests that with feeding strategies reducing enteric CH₄ emissions, it is important to ascertain whether downstream emissions from manure storage partly alleviate the effect on enteric emissions. A GHG balance for feeding and manure storage will be presented.

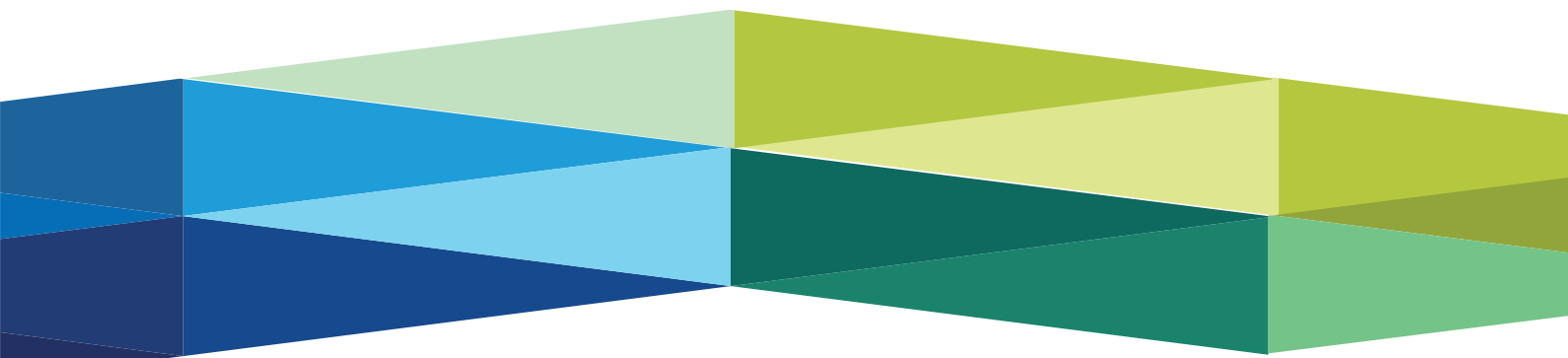


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Theme 5: Greenhouse gas emissions from agricultural soils and grazing systems

5.1 Climate-smart soil and grazing management



97 Plantain to reduce N₂O emissions from pasture-based livestock systems: seven years of New Zealand research

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In pasture-based livestock systems, urine deposited by grazing animals is the largest source of nitrous oxide (N₂O) emissions. Pasture plants typically need a lot more nitrogen for optimal growth than animals need for their protein synthesis, and grazing ruminants generally only utilise between 10 and 25 percent of the nitrogen they consume. The remainder is voided in the animals' excreta, mainly in small urine patches that contain high concentrations of nitrogen.

International and New Zealand-based research has shown that alternative forages can alter the quantity of nitrogen in urine. In addition, research has demonstrated that some plants help to reduce N₂O emissions by influencing the soil microbial processes that produce N₂O, e.g. through biological nitrification inhibition (BNI).

A New Zealand research programme, aimed at quantifying the potential of plantain (*Plantago lanceolata*) to reduce N₂O emissions from grazed systems, has been underway since 2018. This programme has tested various hypotheses on the effect of plantain on N₂O emissions, including i) the reduction in N₂O emissions is linearly related to the proportion of plantain in the pasture swards; ii) the ability of plantain to reduce N₂O emissions varies between plantain cultivars; iii) a high soil nitrogen (N) status reduces the capacity of plantain to reduce N₂O emissions as it limits BNI; and iv) soil type affects plantain's BNI capacity. The N₂O methodologies used included soil incubations, glasshouse trials under controlled conditions and field studies using soil chamber methodologies to measure the effect of varying proportions of plantain on N₂O emissions from animal urine.

Some trials have suggested that pastures containing c. 45 to 60% plantain could reduce N₂O emissions from urine patches by up to 50% compared to urine deposited on ryegrass pasture soil. This was due to both a reduction in the amount of N deposited in urine patches and total amount of daily-N excretion, as well as a reduction in the N₂O emission factor, the latter potentially through BNI. However, other trials have yielded inconsistent results on the relationship between plantain proportion in the sward and N₂O emissions. Our results to-date showed some evidence that the efficacy of plantain to reduce the N₂O emission factor for urine was higher when soil field capacity > 0.45 mm/mm, and also at urine N application rates of 400- 600 kg N/ha. Variability also existed between different plantain cultivars, and between soil types. Soil N status did not affect plantain's ability to reduce N₂O emissions, with the highest reduction found in plantain swards with the highest N status. The focus of current research is to better understand the factors affecting the observed variability in the effect of plantain on reducing N₂O emissions. This paper will provide a summary of the research programme to-date and elucidate the mechanisms underlying plantain's ability to induce BNI and reduce N₂O emissions.

21 Impact of Land-Use types on GHG Emissions, and Aboveground Biomass in Arid Ecosystem of Nigeria

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Conversion of secondary forest to other land-use Types (LT) have been the driver of biodiversity loss and GHG Emissions (GHH-E). It is established that LT has led to significant alterations in Plant Diversity (PD) and ecosystem health in some Northwestern states of Nigeria. Understanding how these alterations impact GHH-E, Aboveground Biomass (AB), can provide valuable insights for sustainable land-use management practices. However, limited information exists on the effect of LT on GHH-E, AB and Soil Properties (SP) in Jigawa state. Therefore, effects of LT and ecosystems on GHH-E, AB, PD and SP in Jigawa was evaluated.

Effects of LT on GHH-E, AB and diversity were investigated across three established ecosystems: Sahel Savanna (Sh.S.), Sudan savanna (SdS) and Wetland (WI) of Jigawa. One sample plot (100x100m) with three replicates were randomly established within each LT (Agroforestry Plots (AP), Crop Farms (CF), Farm Fallow (FF), Grazing Routes (GR), and Protected Areas (PA) (totaling 15 plots/ecosystem) for assessment of Vascular Plants Species (VPS). Twenty-five 1x1m micro-plots were established within each sample plot (totaling 375 micro-plots/ecosystem) across the ecosystems to study the ground-flora. The VPS with Diameter at Stump Height (DSH) ≥ 5.0 cm were measured for height and diameter; and counted. All VPS with DSH ≥ 2 cm ≤ 4.9 cm were considered as shrubs, measured for height and diameter; and counted. One sample plot was randomly selected in each LT for indirect AB estimation. Ground-flora biomass was estimated using direct estimation method. Plant diversity (H'), richness (D), and evenness were estimated using; Shannon-wiener, Margalef, and Hills equations respectively. Soil samples were collected at: 0-15, 15.1-30, 30.1-45cm depths. Data were collected in dry and wet seasons for two-years. Data were analysed using descriptive statistics, ANOVA and Correlation Analysis.

The AB decreased across LT in the order; PA (7088.94kg/ha), AP (5179.83kg/ha), CF (3710.16kg/ha), FF (2379.13kg/ha) and GR (668.94kg/ha). Ground-flora biomass followed the order (GR 33446.67kg/ha, AP 25899.0kg/ha, CF 18551.0kg/ha, PA 3544.47kg/ha and FF 1189.53kg/ha). The highest AB was recorded in WI (6647.81kg/ha) while ShS had the least (1273.18kg/ha). Composition of VPS and herbs decreased from WI to ShS (64 to 47 and 81 to 38 respectively). The GHH-E was largely impacted by the LT in the sequence; PA (0.5 tCO₂-eq ha⁻¹ yr⁻¹), AP (0.2 tCO₂-eq ha⁻¹ yr⁻¹), CF (0.1 tCO₂-eq ha⁻¹ yr⁻¹), FF (0.5 tCO₂-eq ha⁻¹ yr⁻¹), and GR (-0.2 tCO₂-eq ha⁻¹ yr⁻¹). Species diversity and richness for trees, and herbs, showed significant differences across the LT ($H' = 0.018$ and 0.049 ; $D = 0.007$ and 0.037) ($p < 0.05$). Shrub species richness (0.621) and evenness (0.071) did not differ significantly across LT. Factorial analysis showed significant interactions among LT, SP, and VPD ($p < 0.05$). Positive correlation was observed between Calcium and species abundance (0.981), diversity (0.818) and evenness (0.866). Similarly, between Phosphorus and VPS richness (0.818). Agroforestry appears to be the most promising land-use for promoting AVPB and VPD in Jigawa. There is need for further research to understand the benefits and limitations of LT on forest carbon sequestration in the study area.

168 Performance and methane emissions of dairy heifers on contrasting meadow fescue grazing systems

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Pasture-based systems for dairy heifers offer economic and environmental benefits, but the sustainability of these systems depends on optimizing forage productivity, animal performance, and greenhouse gas emissions. In the context of increasing interest in alternative grazing management practices, this study evaluated the performance and methane emissions of Holstein dairy heifers grazing meadow fescue (*Lolium pratense*) pastures under three management systems: Meadow Fescue + N fertilizer (**MF+N**; 157 kg N/ha/yr.), Meadow Fescue without N fertilizer (**MF**), and Meadow Fescue + Clover (**MF+C**). The experiment was laid out in a randomized complete block design, and was conducted at the University of Wisconsin Marshfield Agricultural Research Station, using nine pastures divided into 10 paddocks each, covering a total of 7 ha. The heifers were rotationally stocked using variable stocking rates and put-and-take animals to maintain a herbage allowance of 2.5 kg dry matter (**DM**) per kg body weight. Variables evaluated were pre- and post-graze herbage mass, nutritive value, stocking rates, gain per area, nitrogen utilization and enteric methane emissions. Methane emissions were measured using three GreenFeed systems. Dry matter intake (**DMI**) was measured using TiO₂ as an external marker. There were no significant differences in DMI and average daily gain (**ADG**) among treatments, with an overall mean of 6.7 kg DM/day and 0.63 kg bodyweight/day respectively ($P > 0.05$). Stocking rates were greatest for MF+N and MF+C pastures (4 AU/ha [1 animal unit = 300 kg]) compared to MF (3.8 AU/ha; $P = 0.038$). Herbage nutritive value differed significantly among treatments. There was a treatment by time of evaluation interaction for crude protein (**CP**) concentrations ($P = 0.01$), where these increased as the season progressed, ranging from 14 to 17% from May to September, respectively. There was also a treatment by time of evaluation interaction for in vitro organic matter digestibility (**IVOMD**; $P = 0.00134$), however as the season progressed, digestibility decreased, ranging from 79 to 65%, and the decrease was greater for MF. Ruminant nitrogen microbial production was significantly greater from heifers on MF+N pastures (97 g/day) than in MF+C (74 g/day) and MF pastures (67 g/day; $P = 0.0275$). Efficiency of crude protein utilization (g CP/kg organic matter digested) was also greater in MF+N ($P < 0.001$). Enteric methane production did not differ among treatments, averaging 197 g CH₄/day per heifer ($P = 0.9878$). Methane intensity, expressed as g CH₄ per kg of gain and g CH₄ per kg of dry matter intake also showed no significant differences ($P > 0.05$) averaging 309 g and 32.5g respectively, suggesting that clover inclusion did not increase methane emissions compared to N-fertilized or control pastures. The MF+N treatment demonstrated no substantial differences from the other treatments in terms of productivity or methane emissions, indicating that well-managed systems using legume integration (MF+C) or unfertilized pastures (MF) can achieve comparable outcomes.

217 Carbon footprint and carbon balance of pasture-based dairy systems with contrasting intensification levels in Uruguay

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The intensification of dairy production is essential to meet the increasing global demand for milk and dairy products. However, this process raises environmental concerns, particularly in relation to greenhouse gas (**GHG**) emissions. This study aimed to assess the carbon footprint (**CF**) and carbon balance (**CB**) of two contrasting pasture-based dairy systems representing sustainable intensification strategies: **OS** (Operational Simplicity), with a stocking rate (**SR**) of 1.8 LU/ha, self-sufficient on forage production and based on lower external inputs; and **HP** (High Productivity), with a SR of 3 LU/ha, relying on higher external inputs and designed to maximise productivity. Both systems were evaluated over one fiscal year at the CRS research station (Faculty of Agronomy, UDELAR, Canelones, Uruguay). Total GHG emissions per hectare (E/ha; t CO₂eq/ha) and CF were estimated using IPCC Tier 2 (2019) methodology, while soil organic carbon (**SOC**) sequestration was calculated following Ricard and Viglizzo (2020). Preliminary results show comparable CF values: 0.66 kg CO₂eq/kg FPCM for OS and 0.72 for HP. SOC sequestration contributions were also similar (0.16 and 0.17 kg CO₂eq/kg FPCM for OS and HP, respectively), leading to CB values of 0.50 and 0.55 kg CO₂eq/kg FPCM for OS and HP, respectively. However, total GHG emissions per hectare were substantially higher in the HP system (3.589 vs. 6.278 t CO₂eq/ha), reflecting its higher SR. These results highlight a key trade-off in sustainable intensification: While both systems show potential for achieving productive efficiency with relatively low emissions per unit of milk, the greater intensification level of HP led to higher land-based emissions. Consequently, challenges at larger scales could arise, particularly in the context of land-use planning and climate mitigation targets. Ongoing research is expanding this analysis across three fiscal years to capture interannual variability and system resilience.

254 Developing nitrous oxide emission factors for urine and dung of Chilean grazing dairy cows

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In Chile, livestock represents a significant source of greenhouse gas emissions, with nitrous oxide (N_2O) being one of the main gases emitted by the agricultural sector. Currently, N_2O emissions derived from grazing animals are estimated using the tier 1 methodology based on the 2019 Refinement to the 2006 IPCC guidelines, which proposed a default emission factor of 0.004 kg of N_2O -N kg N^{-1} deposited urine and dung nitrogen (**N**). However, this may not adequately reflect local soil and climatic conditions affecting N_2O production, particularly in southern Chile where livestock production is located. This study aims to determine a country specific N_2O emission factor for urine and dung of dairy cows grazing pasture in autumn and spring.

The study was conducted between April 2024 and March 2025 (in process) at the INIA Remehue Experimental Station (Osorno, Chile), on a typic hapludand soil from the Osorno series with perennial ryegrass variety Nui. Data included in this abstract corresponds to the results of the autumn season, but at this conference, data obtained from both autumn and spring will be presented and discussed. In the autumn trial, urine was applied at 1108 kg N ha⁻¹ while dung was applied at 886 kg N ha⁻¹. A control (no N) was included. Urine and dung were collected from grazing dairy cows at the experimental station and stored at 4°C until application. The treatments were applied with six replicates in a randomised block design. The N_2O emissions were measured by collecting gas samples from static PVC chambers, followed by storage in glass vials prior to gas chromatography. Measurements were taken before and after application at defined intervals over 150 days per season. The N_2O flux was calculated from the slope of the linear increase in its concentration during the closed period, corrected for temperature, headspace volume, and covered area. Nitrous oxide emission factor was expressed as a percentage of applied N. Statistical analysis was performed using mixed models in SAS, with significance $p < 0.05$.

In the autumn trial, cumulative emissions observed in the urine treatment were significantly higher than those observed in the dung treatment ($P < 0.01$), with measured losses of 0.87 kg N- N_2O ha⁻¹ (± 0.107) for urine, 0.40 kg N- N_2O ha⁻¹ (± 0.048) for dung, and 0.22 kg N- N_2O ha⁻¹ (± 0.015) for the control. This means that the accumulated emissions from the urine and dung treatments were 3.9 and 1.8 times higher, respectively, than the control. The N_2O emission factor was 2.8 times higher for urine compared to dung (0.059 and 0.021%, respectively). The significance of these results is reinforced by the 85-95% reduction in the emission factor for the autumn period compared to the default value established by the 2019 Refinement to the 2006 IPCC. In conclusion, urine and dung emission factors differ significantly and should therefore be accounted for separately in the national inventory. Furthermore, these findings contribute to enhancing the accuracy of emission estimations, and better informing resource allocation and public policy development.

238 *In vitro* gas and methane production from chicory, plantain, and birdsfoot trefoil across regrowth weeks

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The objective was to investigate the *in vitro* gas production, CH₄ production, and fermentation characteristics of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and birdsfoot trefoil (*Lotus corniculatus*) at different weeks of regrowth. Adopting a 3 (forage species) × 5 (weeks of regrowth) factorial design, we collected plant samples in August and September 2023, from three independent plots (2.0 m × 0.6 m) in the field after two to six weeks of regrowth (weeks 1 to 5). These 45 samples were analysed for chemical composition and incubated in fermentation flasks in triplicate with mixed rumen fluid from three lactating rumen-cannulated Holstein-Friesian cows in three runs. *In vitro* gas production was measured over 48 hours using an automated gas production system with CH₄ measured at distinct time points. Organic matter degradability, pH, ammonia concentration, and volatile fatty acid composition were determined at the end of incubation. Per incubated sample data from triplicate flasks were averaged and analysed using a mixed-effects model, with species, regrowth week, and their interaction as fixed effects, and run as a random effect. Interactions between species and regrowth week were observed for organic matter degradability, as well as for gas and CH₄ production (mL/g organic matter incubated) ($P < 0.05$). Chicory exhibited higher organic matter degradability than plantain and birdsfoot trefoil, with the differences narrowing from weeks 1 to 4 before widening in week 5. Chicory also showed greater gas production (mL/g organic matter incubated) than the other species, although the differences diminished during the final two regrowth weeks. However, gas production (mL/g organic matter degraded) was only affected by species ($P < 0.05$), with a greater gas production of plantain than of chicory and birdsfoot trefoil. Plantain also exhibited lower CH₄ production (mL/g organic matter incubated) compared to chicory in regrowth weeks 1 to 3, but did not differ from birdsfoot trefoil. When expressed per unit of degraded organic matter though, CH₄ production was only affected by species ($P < 0.05$), with a lower CH₄ production of chicory than of plantain and birdsfoot trefoil. These results are more likely attributable to non-tannin plant secondary metabolites rather than to differences in chemical composition. In conclusion, chicory exhibited greater organic matter degradability and gas production relative to organic matter incubated, but lower CH₄ production relative to organic matter degraded, than plantain and birdsfoot trefoil, although the differences often varied across regrowth weeks.

133 Effect of access to production pasture versus exercise pasture on dairy cow methane emissions

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In northern Europe dairy cows are predominantly confined to indoors rearing. Animal welfare legislations in Scandinavian countries require outdoor access for cattle at minimum during growing season. Economic pressures have forced an increase in average herd sizes and production yields. This requires larger areas for preserved feed production, and farm-close land has become a limitation for providing pasture as a significant feed source. Consequently, outdoor access is often limited to function as a measure of exercise- and leisure. Dairy cows in pasture-based production systems have demonstrated less GHG emissions, when compared to cows with exclusively indoor feeding. However, the effects of seasonal access to pasture have been less vigorously studied and this knowledge-gap may lead to an overestimation of GHG emissions from the Nordic dairy industry.

The aim of this experiment was to assess effects on enteric methane emissions, milk production and -composition when offering dairy cows voluntary access to pasture. We hypothesized that cows milked by AMS with an unrestricted herbage allowance during the grazing season have similar animal performance, but lower methane emissions compared to cows offered outdoor exercise only.

In a change-over design, 32 Norwegian Red dairy cows were blocked into groups of 16. Each group had 24-h access to either a production pasture or an exercise paddock. Cows in the production pasture treatment were given an ad libitum herbage allowance but were limited on their indoor grass silage ration. Cows in the exercise treatment were offered an outdoor area with a highly limited herbage availability and an ad libitum silage ration indoors. Indoor feed intake was recorded by access-controlled feed bunks and concentrate feeders, milk yield in the AMS, and enteric methane emissions in two GreenFeeds – one located indoors and one by the production pasture entrance. Pasture intake was calculated by difference; estimated energy requirements for maintenance, lactation, gain and activity, minus recorded indoor energy intakes. A mixed effect ANOVA was performed using R.

Cows on production pasture had 51% of their daily dry matter intake from grazed grass, 25% from grass silage and 24% from concentrate. In contrast, cows in exercise treatment had 62% dry matter intake from grass silage. Overall, total dry matter intake was similar between the treatments. Cows in the production treatment exhibited a lower milking frequency and reduced milk volume compared to cows on the exercise treatment. However, cows on production treatment compensated for their lower milk volume by an increased milk solids concentration, suggesting that incorporating a significant amount of pasture in the diet of dairy cows may not compromise energy-corrected milk production. Additionally, cows in the production treatment had significantly lower absolute methane emission, methane yield and -intensity, compared to cows in the exercise treatment. A possible contributing factor for this reduction is a lower NDF content and a higher digestibility of the fresh grass.

Our results suggest a potential reduction in the methane emissions of dairy production by including a significant herbage component in the diet of dairy cows also where pasture access is restricted by season.

129 Can soil and tree carbon sequestration maintain zero net emissions grazing?

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Goals, set in response to The Paris climate agreement (**COP21**) have created an imperative for livestock producers to reduce greenhouse gas emissions (**GHGe**) where possible. However, methods to reduce emissions in livestock enterprises are limited. The primary pathway to reduce the balance of GHGe produced on farms is through utilising farmland to sequester carbon in soils and trees within the farm boundary, removing GHGe from the atmosphere. To assess the effectiveness of soil and tree carbon sequestration in maintaining net zero emissions on Jigsaw Farms, a grazing enterprise comprised of two properties; Hensley Park and Melville Forrest, who achieved net zero status in 2014. To calculate the net emissions produced by Jigsaw Farms, the volume of GHGe produced and sequestered in 2021 was determined. On-farm and pre-farm emissions were calculated using the IPCC-approved method, the SB-GAFv2.3. FULLCam was used to estimate carbon sequestration in trees and shrubs. Both emissions and sequestration results were validated through field data and farm records. From 2010 to 2017 Jigsaw Farm's Hensley Park property sequestered a greater volume of carbon in trees and soils, than it produced. Jigsaw Farms however is no longer a net zero enterprise due to an increase in GHGe from higher animal numbers, and a reduction in sequestration as trees reached maturity, and carbon saturation. In 2021, Jigsaw Farms produced 10,870 t CO₂-e in Scope one, two and three emissions, and sequestered 6,704 t CO₂-e across both Hensley Park and Melville Forrest. Although Jigsaw Farms did not maintain net zero emissions in 2021, the net balance of emissions (4,166 t CO₂-e) resulted in the emission intensity of Jigsaw's wool, lamb and beef products, more than halving. Providing suppliers and consumers with a low emission livestock product. Expanding tree plantings, using faster-growing species, or incorporating harvested timber in long-lived products could extend zero and low emission periods. However, trees cannot indefinitely balance emissions as they do not address the cause of emissions. Ultimately net zero livestock production requires innovations that reduce enteric methane, to sustainability achieve and maintain net zero emissions in livestock systems.

85 The effect of management practices on N₂O emissions from a northern mineral soil agricultural field

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Agricultural soils are significant sources of N₂O, as they have been estimated to be responsible for 60% of global anthropogenic N₂O emissions. Majority of these emissions are due to application of synthetic nitrogen (N) fertilizers. Globally 120t of new N is introduced to soil every year to sustain crop and grass production. In Finland, agricultural sector produces 13% of the total GHG emissions.

Growing season in Finland is relatively short (May-September), and all grazing and animal feed production takes place during this period. In Finnish agriculture, harvested grass is usually baled to enable the preservation of feed into the winter, for indoor feeding of animals. Agricultural fields used exclusively for forage grass production are typical in Finland, and about 35% of the country's arable land is cultivated with forage. With optimized management practices, such as crop species selection and timing of fertilization, N₂O emissions from agricultural fields and per units of feed produced could be reduced. Since N₂O is the most potent GHG, even small reductions in its emissions can yield to significant climate benefits.

Introducing legumes, such as red clover (*Trifolium pratense*), to crop rotations reduces the need of synthetic fertilizers, due to the ability of the legumes to fix their own N via symbiosis with rhizobia bacteria. Clover fodder, mostly used on dairy farms, is particularly valued for its high protein content. Previous studies have shown lower N₂O fluxes in red clover grass mixtures compared to monocultures and grass mixtures with other grass species. Lower levels of synthetic N fertilization also reduce indirect N₂O and CO₂ emissions, which are generated during the fertilizer manufacturing process. In mineral soils, highest N₂O emission peaks are often measured after fertilization events. The fertilizer induced emission peak can be reduced by delaying fertilization, e.g. a week after harvest, when plants are actively growing and are able to utilize the nutrients.

In this research we aim to answer to two research questions: 1) Does delaying the fertilizer application after harvest result in lower N₂O emission peaks? 2) Does red clover coverage reduce annual N₂O emissions from agricultural fields to the atmosphere? The research was conducted in a 6.3ha agricultural field on a mineral soil, in Maaninka, eastern Finland. The field is used exclusively for producing baled feed for animals. We studied the N₂O exchange of the field using the eddy covariance technique during three years of grass rotation cycle (2022–2024). Plant, soil and environmental variables were also measured to help explain the N₂O exchange patterns and N dynamics.

This presentation describes the N₂O exchange trends as affected by different management practices. We hypothesized that delaying the fertilizer application decreases the N₂O emission peaks, and that annual N₂O emissions from the agricultural field decreases with red clover coverage. Highlighted are the changes in red clover coverage, total yield, C and N in yield, as well as the effect of delayed fertilization, and the annual dynamics of N₂O. The research will be completed, and the results will be shared at the conference poster.

300 Impacts of management on soil carbon sequestration and ecosystem resilience in Kenya's semi-arid rangelands

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Grasslands play a critical role in providing livelihoods, global ecosystem resilience, soil carbon (C) storage, and climate mitigation. However, they are increasingly threatened by Mal grazing, land fragmentation, invasive species, and climate change. Sustainable management strategies offer pathways to maintain grassland functionality, protect soil C, and provide forage and habitat for livestock and wildlife. However, there is limited information on the soil C storage potential of semi-arid grasslands in sub-Saharan Africa. This knowledge gap hinders our ability to provide evidence-based recommendations to livestock keepers and land managers for maintaining soil health in rangelands, and it also causes uncertainty in global carbon models. Our study investigates the impact of grassland management on soil health, nutrient cycling, greenhouse gas (GHG) emissions, and carbon stocks in three study sites in Kenya: ILRI's Kapiti Research Station in Machakos County, the Mukutan Conservancy in Laikipia County, and Meru National Park in Meru County. Paired grassland plots were selected along fencelines and natural borders and we sampled both inside the protected area (conservancies and national parks), which served as the natural baseline, and outside protected areas in the (often) degraded communal grazing lands. We sampled between 8 and 11 paired plots at each study site, where we dug soil profiles for soil sampling and measured GHG emissions, water infiltration, and vegetation cover and biodiversity (n=3 per plot). Soil samples are currently being analyzed for soil organic C (SOC), total and mineral nitrogen (N) and phosphorus (P), pH, texture, aggregate stability, microbial biomass (C and N), and extracellular enzyme activity. Vegetation samples are being analyzed for crude protein (CP), fiber and metabolizable energy content, and plant biodiversity indicators. Furthermore, we are currently conducting a laboratory incubation using soil cores collected from the three sites to estimate the GHG emission potential. Our aim is to determine the soil carbon sequestration potential of these grassland soils by comparing the natural baseline with outside plots, and to improve our understanding of nutrient cycling, GHG emissions, and vegetation dynamics across communal livestock grazing areas and wildlife conservation zones. Through this, we seek to provide robust data to guide sustainable land-use practices and inform biogeochemical modelling. Ultimately, this research has the potential to inform policies and management strategies that promote soil health, enhance carbon sequestration, and improve the resilience of grassland ecosystems in Africa.

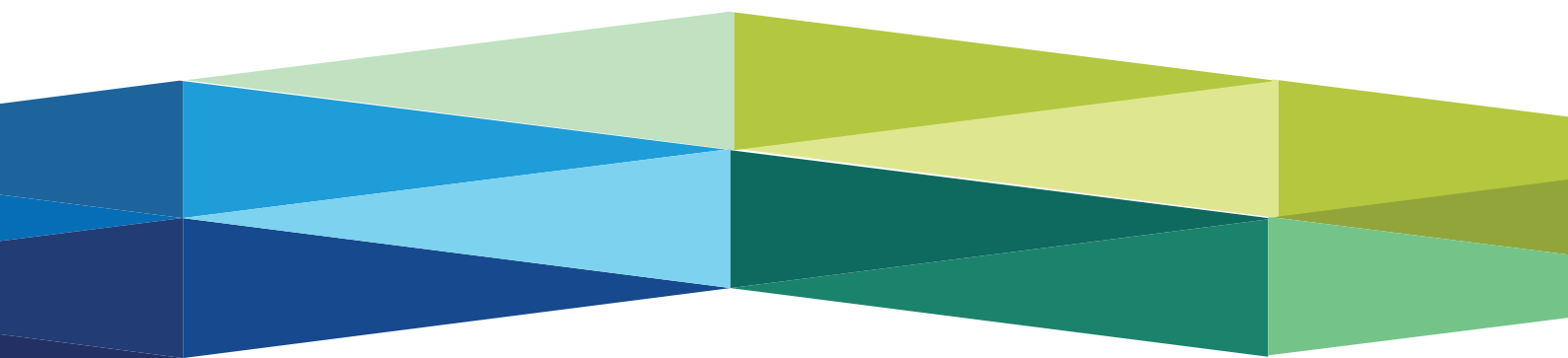
The study is part of the EFP-Soil funded CarboGrass project, which is an international consortium comparing grassland carbon stocks and functions across climatic and management gradients in 8 countries.



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Conference

5.2 Global approaches to mitigating GHG emissions in agricultural soils and grazing systems



388 The first measurements of methane and milk production from Fijian dairy cows

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The Fijian dairy industry faces the challenge of balancing economic growth and food security, with environmental sustainability. Fijian dairy herds are considered to have low production, resulting in high greenhouse gas (**GHG**) emission intensity. The objective of our study was to begin quantifying the milk production and enteric methane characteristics of Fijian dairy cattle.

There were four Brown Swiss cows 108 ± 22.0 (mean $\pm$ standard deviation) days in milk and weighing 480 ± 14.2 kg; and six Kiwi Cross cows 163 ± 64.8 days in milk and weighing 435 ± 50.7 kg were enrolled in the experiment at the Koronivia Research Station, Nausori, Fiji. Cows were offered a common diet of concentrate (7.5 kg DM day⁻¹), fresh cut and chaffed Elephant grass (0.7 kg DM day⁻¹), and fresh cut pasture (4.7 kg DM day⁻¹). Feed was offered half in the morning and half in the afternoon in individual stalls. After feeding, cows were moved to a yard for loafing where water was available ad libitum. Cows were milked at 05:00 and 15:00. Methane was measured using the SF₆ technique, as implemented for local conditions.

There were no statistical differences in dry matter intake, energy corrected milk (**ECM**) yield, methane production, methane yield, nor methane intensity between Brown Swiss and Kiwi Cross cows. However, numerically, the results show an alternative perspective. Yield of ECM of the Brown Swiss cows was 15% greater than from the Kiwi cross cows (11.0 vs. 9.6 kg d⁻¹, $p = 0.29$), similarly for feed conversion efficiency (0.85 vs. 0.74 kg-ECM kg-DMI⁻¹, $p = 0.27$). Methane production of Brown Swiss cows was 9% greater than that from the Kiwi Cross cows (282 vs. 259 g d⁻¹, $p = 0.42$), as was methane yield (21.8 vs. 20.1 g CH₄ kg-DMI⁻¹, $p = 0.42$) once outliers were removed. Methane intensity was 4% higher for Brown Swiss cows than Kiwi Cross cows (25.8 g kg-ECM⁻¹ vs. 26.8 g kg-ECM⁻¹, $p = 0.68$).

Milk production and methane parameters were successfully measured. However, the large numerical differences were not statistically different, indicating that results from more cows need to be added to the data pool before firm conclusions can be drawn around milk production and methane outputs of Fijian dairy cows.

393 Improved pastures for soil carbon sequestration in tropical forage-based systems to mitigate greenhouse gas emissions.

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Tropical pasture-based livestock systems play a crucial role in global food production, but they also contribute significantly to greenhouse gas (GHG) emissions. Sequestering carbon (C) in soil organic matter in pasturelands is considered as a key strategy to offset GHG emissions. While improved pastures contribute to reducing ruminant methane (CH₄) emissions per unit livestock product, their capacity to sequester soil C is still to be explored broadly. This study aimed to explore the potential of improved pastures for soil C sequestration in tropical forage-based systems to help developing mitigation strategies and enhancing the sustainability of these systems. We applied the Sustainable Grazing Systems (SGS version 5), a biophysical pasture simulation model, to simulate five different long-term pasture production systems comprising two native tropical pastures (native C4 and native low-quality C4) and three improved pastures: *Brachiaria ruziziensis* x *B. decumbens* x *B. brizantha* 'Brachiaria Mulato II' (BM), *Megathyrsus maximus* 'Gatton Panic' (GP), and *Chloris gayana* 'Rhodes grass cv. Reclaimer' (RR). Soil parameters (initial organic and inorganic matter and soil physical and water parameters) were obtained from the SRICALSOL database. Daily climate data (rainfall, solar radiation, and minimum and maximum temperatures) were sourced from the AgMERRA database. Five separate simulations were carried out in three agro-climatologically distinct livestock regions: Dry Zone (Polonnaruwa), Intermediate Zone (Kurunegala), and Wet Zone (Peradeniya) in Sri Lanka over a 30-year (1980-2010) period under the business-as-usual scenario management inputs (rainfed and low nitrogen fertiliser, monthly cut-and-carry). The model simulated annual soil C balance (t C ha⁻¹) was extracted and converted to CO₂eq by multiplying by -3.67. Results showed that the annual change in soil C was influenced by the region (soil and climate) and pastures and region × pasture interactions (p<0.05). Mean annual soil C was variable, ranging from an annual sink in the Wet Zone (0.43 t CO₂eq) to an annual emission of 2.35 t CO₂eq and 1.79 t CO₂eq in the Dry and Intermediate Zones, respectively. Both native and native low-quality pasture systems showed higher emissions, ranging from 2.40 t CO₂eq to 2.58 t CO₂eq, respectively. Among the three improved pastures, BM acted as a C sink, sequestering an average of 0.30 t CO₂eq year⁻¹, while RR and GP systems emitted a mean value of 0.24 t CO₂eq and 0.97 t CO₂eq year⁻¹, respectively. The study demonstrated that the use of improved pastures in tropical forage-based systems can significantly enhance soil C sequestration. However, the implications of greater productivity of improved pastures and associated greater carrying capacity and emissions of GHG such as CH₄ and N₂O need to be further investigated within a whole-farm context to understand the net GHG balance.

169 A life cycle assessment of dairy heifer rearing systems in Wisconsin

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This study employs a life cycle assessment (**LCA**) to evaluate greenhouse gas (**GHG**) emissions and milk production efficiency of eight dairy heifer-rearing systems common to Wisconsin. The systems were: (1) conventional confinement with average milk yield, (2) out-of-state dry lot with average milk yield, (3) pasture-based without supplementation and with increased milk yield, (4) pasture-based with supplementation and with increased milk yield, (5) pasture-based without supplementation and with average milk yield, (6) pasture-based with supplementation and with average milk yield, (7) conventional confinement with increased milk yield, and (8) out-of-state dry lot with increased milk yield. The GHG emissions were analyzed during the heifer-rearing and first lactation phases to identify strategies for optimizing productivity while minimizing environmental impacts. Average milk yield was considered to be 37.5 kg/day, while increased milk yield was defined as 42 kg/day. Methane (**CH₄**) from enteric fermentation emerged as the largest contributor to GHG emissions across systems, accounting for 47% in confinement systems and 52% in pasture-based systems. Methane from feces contributed 24%, while feed-related emissions accounted for 21% of total emissions. Systems 7 and 8, confinement-based systems with increased milk yields, exhibited the greatest total emissions (1.3 million kg CO₂e), primarily due to energy-intensive feed production and manure management. Despite their greater milk yields, these systems had moderate emission intensities compared to other systems. System 3, a pasture-based system without supplementation but with increased milk yield, achieved the least emission intensity (1.42 kg CO₂e/kg of milk) and relatively low total emissions (1.19 million kg CO₂e). This system benefited from reduced energy inputs, resulting in minimal CH₄ emissions relative to milk output. System 4, a pasture-based system with supplementation and increased milk yield, had slightly greater emission intensity (1.43 kg CO₂e/kg of milk) but comparable total emissions (1.20 million kg CO₂e). While supplementation reduced enteric methane emissions by promoting propionate production in the rumen, it increased nitrous oxide (**N₂O**) emissions from manure due to the greater protein concentration of the diet and consequent lower C/N ratio in feces. Systems 5 and 6, representing pasture-based systems with average milk yields, had the least total emissions (1.17 million kg CO₂e). However, their emission intensities were greater (1.55 kg CO₂e/kg of milk) due to lesser milk yields, which increased GHG emissions per unit of milk produced. Systems 1 and 2, confinement systems with average milk yields, had similar total emissions to Systems 7 and 8 (1.27 million kg CO₂e) but exhibited greater emission intensities due to their lesser milk productivity (1.69 kg CO₂e/kg of milk and 1.54 kg CO₂e respectively). System 3 demonstrated the best overall balance, achieving the least emission intensity while maintaining low total emissions. System 4 closely followed, with slightly greater intensity but added productivity benefits. Therefore, pasture-based systems, particularly those with efficient grazing management, can significantly lower GHG emissions compared to confinement systems. They offer a sustainable alternative by balancing environmental benefits with productive efficiency.

387 Carbon Uptake in East African Drylands: Insights from four ecosystems

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Semi-arid ecosystems play a pivotal role in regional carbon budgets, yet their responses to climate variability including extremes remain poorly understood. This study examines carbon flux dynamics across four eddy covariance towers located in semi-arid Kenya, representing four different land-use systems: commercial dryland agriculture, a smallholder farm, a savannah grassland, and a wooded savannah rangeland, both of which support livestock grazing and wildlife. While data availability varies across the sites, with 2-5 years of records and gaps in some cases, this study provides preliminary insights into carbon flux dynamics under different seasonal and climatic conditions. We hypothesized that seasonality and climate extremes such as multi-year droughts, lead to significant carbon losses that are not fully compensated during adjacent wet seasons, and thus leading to long-term carbon imbalances. Preliminary analysis reveal that during dry seasons, croplands (typically fallow with exposed soil) experience relatively low carbon losses compared to rangelands, as minimal biological activity results in reduced net ecosystem exchange (**NEE**). In contrast, rangeland ecosystems show higher NEE during dry periods, indicating greater carbon losses driven by persistent ecosystem respiration (**Reco**) and limited photosynthesis (**GPP**). During wet seasons, croplands display rapid and significant carbon uptake (negative NEE) following rainfall events due to fast crop growth and fertilizer use. However, this uptake diminishes after the crops mature, as senescence of the crops reduces photosynthetic activity before harvesting. Rangelands recover more gradually, with a moderate but sustained carbon uptake due to the slower response of native vegetation. Our findings provide insights into how semi-arid ecosystems in East Africa respond to increasing frequency and intensity of climatic extremes. While natural variation in carbon cycling is expected, the intensification of extreme events raises questions about the long-term implications for ecosystem carbon storage.

457 Calibration of the LandscapeDNDC model for nitrous oxide emissions in Sub-Saharan African cropping systems

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Accurate quantification of nitrous oxide (**N₂O**) emissions from smallholder cropping systems in Sub-Saharan Africa (**SSA**) is essential for improving national and regional greenhouse gas (**GHG**) inventories. However, experimental data are expensive and limited. This, together with SSA countries having limited information on agricultural activity data and being unique in terms of small-scale agriculture, leads to significant gaps in emissions estimates at a large scale.

This study addresses this gap by applying the biogeochemical model LandscapeDNDC to estimate N₂O emissions and crop yields across different sites, crops, and bioregions in SSA. The model was optimized using the SPOTPY-LDNDC algorithm and tested against 280 field datasets (site-year-treatment combinations) from 43 locations representing diverse agroecosystems. Through this approach, we evaluate the model's ability to reproduce observed N₂O fluxes and explore key drivers influencing emissions.

Our findings demonstrate that LandscapeDNDC effectively captures the variability in N₂O emissions across different cropping systems, highlighting its potential as a tool for national and regional GHG inventories.

We also outline a conceptual plan based on combining the model with RS-retrieved land use and land management information for developing an automated system for N₂O emission estimation in SSA using the LandscapeDNDC model, which could eventually contribute to a more comprehensive understanding of soil N₂O fluxes from cropping systems in SSA, supporting efforts to develop targeted mitigation strategies and improve emissions reporting.

174 Profitably adapting a cattle and sheep farm business to the cost of CO₂e emissions

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Australian red meat producers generate carbon dioxide equivalent emissions (CO₂e) which cause negative externalities to society. These producers will face the cost of these emissions through direct charges or because of investing to reduce them. This study explores the implications for risk and profit for a case study livestock farm business bearing costs associated with net CO₂e emissions.

The research focuses on a 1000-hectare, high-stocking-rate lamb, and beef production business in South-Eastern Australia. The base case maintained a self-replacing production system for sheep and cattle, with livestock numbers in a 'steady state'. The property had a history of revegetation, with 8% of the area under permanent tree cover, contributing to CO₂e sequestration. Scenarios of different farm plans aimed at reducing CO₂e emissions were examined. By modelling the farm business, the study evaluated the impact on profit, cash flow, wealth, and risk over a 12-month production cycle. Key metrics included annual Net Cash Flow, Return on Capital, and Return on Equity. The analysis utilized the Sheep and Beef Greenhouse Accounting Framework (**SB-GAF** version 2.3) to incorporate CO₂e emissions data and followed IPCC guidelines. The risk associated with various farm plans was incorporated using stochastic modelling; the standard deviation around the mean of annual profit and net cash flow indicated the combined business and financial risk. In the analysis it was assumed emissions have a cost, with each plan having two carbon price scenarios (\$50 and \$100/tonne CO₂e). The farm emission costs are shared between producers and consumers. The contrast between technical efficiency criteria of emissions Intensity associated with a unit of output, and economic efficiency indicated by total emissions and farm profit was revealing.

The first set of adaptation strategies analysed included modifying animal finishing methods, altering pasture and animal genetic potential, plus implementing innovations like a cattle vaccine to mitigate total farm emissions while intensifying the operation for enhanced profitability.

The aim was to see if it was possible to run the case farm business profitably with acceptable risk after paying the net CO₂e emissions cost. A second set of adaptation options reducing the stock numbers and other inputs was also examined. The analyse revealed that pasture and animal genetic improvements reduced emissions per production unit while increasing total profit after the costs associated with CO₂e emissions were covered. Total risk rose with increased expenditure and profit, while farm net CO₂e emissions rose marginally, with additional profits covering this increasing cost. Reducing stocking rates led to lower net CO₂e emissions but cash flow and profit were insufficient for the business to be viable.

The findings suggest, depending on carbon pricing and the costs associated with emissions reduction, the case study livestock producers may be able to increase profits by implementing a mix of emission-reducing and profit-increasing strategies. Doing so increases the total risk.

250 Nitrous oxide emissions from dairy cattle urine and its relationship with milk urea nitrogen content

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Nitrous oxide (**N₂O**) emissions from livestock, especially dairy cattle, represent a significant environmental challenge due to their high global warming potential. In Uruguay, more than 80% of N₂O emissions in agriculture stem from livestock urine and feces. The relationship between nitrogen excretion in urine and its potential to contribute to N₂O emissions is not fully understood. This study aimed to explore whether milk urea nitrogen (**MUN**), a common measure on dairy farms, can serve as a reliable indicator of nitrogen (**N**) excreted via urine and its subsequent conversion into N₂O. Additionally, the study sought to quantify N₂O emissions from urine with varying nitrogen concentrations and assess their relationship to the nitrogen content in the urine and the corresponding soil transformations. To achieve these objectives, the study was conducted in two phases. First, urine samples were manually collected from milking dairy cows at two dairy farms (INIA La Estanzuela and CRS Dairy Unit) in Uruguay between December 2021 and March 2022. Along with the urine samples, MUN values were also recorded for each cow, allowing for an analysis of the correlation between MUN levels and urinary nitrogen content. The second phase of the study involved a controlled experiment where different concentrations of nitrogen (1.43, 4.28, and 5.71 g N/dL) were added to soil in static closed flow chambers. This setup allowed for the measurement of N₂O emissions from urine and the monitoring of changes in soil nitrogen, specifically ammonium (**NH₄⁺**) and nitrate (**NO₃⁻**) concentrations.

The findings showed a weak but positive linear correlation between MUN and nitrogen content in urine, suggesting that higher MUN values are associated with higher nitrogen excretion through urine. Furthermore, the study demonstrated that higher nitrogen concentrations in urine led to significantly higher N₂O emissions. Specifically, urine with higher nitrogen content (N₂ and N₃ treatments) generated the greatest N₂O emissions compared to the control, which had no urine applied. Soil samples from these treatments showed increased NH₄⁺ concentrations, which gradually transformed into NO₃⁻, a process that was linked to the observed N₂O emissions. These results indicate that urinary nitrogen content, influenced by MUN levels, plays a crucial role in the magnitude of N₂O emissions from agricultural soils. This study concludes that MUN can be used as an effective proxy to estimate urinary nitrogen excretion in dairy cattle, offering a practical tool for managing nitrogen intake and reducing N₂O emissions. By adjusting the crude protein content in cow diets based on MUN values, dairy farmers can potentially minimize nitrogen losses and mitigate their environmental footprint. These findings contribute to the development of more sustainable dairy management practices aimed at reducing agricultural greenhouse gas emissions.

110 Carbon sequestration and soil health in integrated crop-livestock system on sandy soil in Brazil

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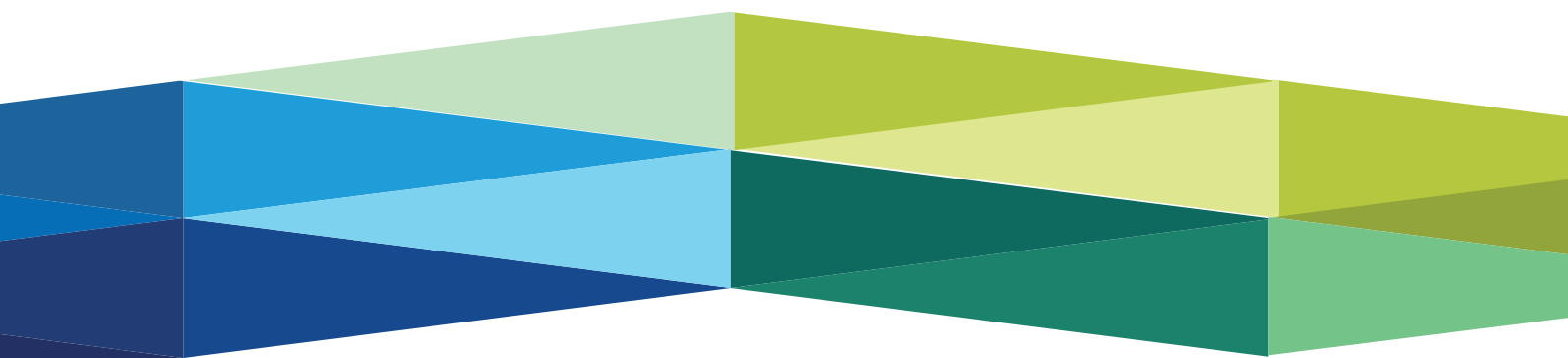
Brazil has approximately 100 million hectares of pastures with medium to low vigor, a situation that is even more critical in sandy soils due to their chemical, physical, and biological fragility. To address this problem, the Brazilian government issued a decree aiming to restore around 40 million hectares of degraded pasture by 2023, and the introduction of integrated systems has emerged as a viable technology to achieve this goal. In 2019, a research initiative was launched by Embrapa at Fazenda Trijunção, located in western Bahia, Brazil, to study the management of soil, plant, and animal components through the implementation of the crop-livestock integrated (**ICL**) system. For the soil component, samples were collected at depths of 0–10 cm, 10–20 cm, and 20–40 cm to measure carbon sequestration (**C**) and the soil health index (**SHI**) (specifically in the 0–10 cm layer). The study evaluated the ICL system against a Conventional (**Conv**) system as a control (low input) and the natural Cerrado (**Cer**) as a baseline. Carbon sequestration was found to be higher in the ICL system (13.55 and 11.77 Mg ha⁻¹) compared to the Conventional system (10.89 and 9.48 Mg ha⁻¹) and to the Cerrado (8.64 and 9.61 Mg ha⁻¹) in the 0–10 cm soil layer for 2019 and 2023, respectively. In the 10–20 cm soil layer, no significant differences were observed among the three systems. However, in the 20–40 cm soil layer, the Cerrado exhibited greater carbon sequestration than the ICL and Conventional systems only in 2023, with no significant differences observed between the latter two. No significant differences were observed between 2019 and 2023 within each system. The Soil Health Index (**SHI**) calculated for the ICL and Conv systems was higher in the ICL system compared to the Conv system, with an improvement observed between 2019 and 2023. This improvement led to a transition in soil health under the ICL system, moving toward a healthier condition during this period, while the Conv system showed a decline in soil health over the same timeframe. The higher forage and meat production observed in the ICL system (data not presented in this study) further supports the potential of integrated systems as effective solutions for restoring degraded pastures. That technology is more sustainable and environmentally friendly way, while also reducing greenhouse gas emissions, deforestation, and boosting income for farmers.



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Theme 6: Circular economy



331 Lessons learned while implementing a technology to mitigate methane emissions for the beef cattle industry

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Domestic ruminants produce large amounts of methane (**CH₄**), which originates from enteric fermentation (**EF**). According to the U.S. Environmental Protection Agency, in 2018, the global CH₄ production from EF represented 28% of the CH₄ emitted globally by the agricultural, forestry and other land-use sectors. The beef industry and a few fast-food companies are aware of this problem. In 2018, a global restaurant company and its associate creative agency asked our scientific group to share and demonstrate a technology for mitigating CH₄ emissions that could be transferred and implemented by their beef suppliers. This company also wanted to be perceived by consumers as environmentally friendly industry. The proposed technology had to reduce EF CH₄ production at a low cost without affecting animal health and productivity, be sustainable, provide co-benefits to cattle farmers and the fast-food sector, contribute to the conservation of the environment, and not introduce toxic substances to the human food chain. We proposed supplementing *Cymbopogon citratus* (lemongrass, **LG**) to fattening beef cattle. LG is a perennial grass cultivated in sub-tropical and tropical regions of the world. Its diverse and rich bioactive compounds in LG, such as phenols, saponins, tannins, flavonoids, alkaloids, steroids, anthraquinones, and high concentration of essential oils, made it a good candidate for reducing rumen CH₄ production because all these compounds have demonstrated anti-methanogenic activity. We conducted several experiments in open-circuit respiration chambers to evaluate the anti-methanogenic potential of different supplementation levels of LG on beef cattle. The results of the experiments suggest that supplementing 100 g of LG DM/head/day can reduce by up to 33% CH₄ yield (g CH₄/kg of food expressed on a dry matter basis, DM), $P < 0.05$, in beef cattle, fed a finishing diet high in concentrates. LG also reduced by 26% the daily CH₄ emission ($P < 0.05$) of cattle fed a diet high in forage, and there is some evidence that live weight increases, too. In 2020, the company made these results public in different world media outlets, e.g. BBC, CNN, CBS, etc. Farmer's associations were unhappy about the media campaign because they believed it exposed them as an important source of greenhouse gases, and some scientists raised criticism, too. Furthermore, the fast-food company did not know how to implement and disseminate the new technology among its beef suppliers. Lessons learned: The CH₄ emission from cattle is a delicate issue for farmers as they do not want to be exposed as a significant source of GHG because it adds an extra burden to an already complicated productive activity. It is concluded that we need to educate farmers about the role of cattle in anthropogenic CH₄ emissions because their participation is essential to achieving mitigation. At the same time, farmers need to receive financial benefits from mitigation. We also learned that there is little beef industry experience in mitigating CH₄ emissions. Scientists need to produce ready-to-use technology to facilitate adoption, and avoid consumers' setbacks, e.g. Britain's largest dairy co-op found itself in the midst of consumer backlash over plans to trial 3NOP.

361 Evaluating greenhouse gas abatement, nitrogen efficiency and cost-effectiveness of circular practices in UK farming

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Transitioning to more circular farming systems that prioritise renewable and recycled resources is a relatively underexplored adaptation and mitigation strategy. However, it offers significant potential to enhance nutrient cycling while reducing embedded emissions and farm costs associated with external inputs such as fertilisers, feed and bedding. We evaluated five circularity indicators for measuring the impact of circular practices on nutrient cycling and efficiency. It also assessed the potential and cost-effectiveness of these practices to reduce greenhouse gas emissions in UK livestock systems, contributing to the UK goal of achieving net zero by 2050. Average regional farm data were used to estimate nitrogen inputs, internal flows and outputs for five farm types (mixed, arable, dairy, dairy-beef and beef-sheep) in the UK. These estimates were the baseline for calculating the farm-level circularity indicators: *farm-gate nitrogen balance*, *nitrogen use efficiency*, *secondary-to-total input ratio*, *cycling index* and *edible protein conversion ratio*. Baseline greenhouse gas emissions were also estimated using the Agrecalc carbon calculator. Eight management practices that potentially increased circularity were developed to evaluate their impact on nitrogen cycling, efficiency, and farm-level emissions using mass flow models. The evaluated practices encompassed on-farm, between-farm and regional strategies, including solid-liquid slurry separation, composting farmyard manure, anaerobic digestion of manure, application of biosolids, alternative feed ingredients, adoption of cover crops and multispecies leys, mixed crop-livestock systems and resource sharing. Cost-effectiveness was assessed using standard farm management cost data and industry benchmarks. Marginal abatement cost curves were constructed to highlight variability in abatement potential and cost-effectiveness across farm types. Significant changes in circularity indicator values were observed when circular practices were implemented individually. Anaerobic digestion had the most substantial impact across livestock farming systems, increasing nitrogen use efficiency by up to 12% and raising the proportion of secondary (renewable and recycled) and internally cycled nitrogen resources by 4%. Anaerobic digestion significantly reduced greenhouse gas emissions but was not cost-effective. In contrast, implementing alternative feed ingredients transformed the mixed farm, housing monogastric pigs and poultry, from an inefficient to an efficient producer of human-edible protein. The edible protein conversion ratio improved markedly, decreasing from 1.71 to 0.66. This practice also emerged as the most cost-effective option for the mixed farm, reducing emissions by 4.7% and saving £200 per Mg CO₂e abated annually through reduced concentrate feed inputs. Our findings highlight the potential of circularity indicators to optimise nitrogen use, add value to waste and by-products, estimate self-sufficiency and farm business resilience, and validate sustainable farming practices and land use. These indicators could be adapted to other critical resources, such as carbon, phosphorus, energy and water, and scaled up to regional and national levels for a more comprehensive evaluation. This study demonstrated the significant potential of circular practices to reduce emissions and enhance cost-efficiency in UK agriculture. There is also great potential to reduce regional-scale impacts and align policies with the broader environmental, economic and social benefits of circular farming systems, supporting their adoption as a key pathway to achieving net zero.

260 Effects of spent coffee grounds on grass silage quality and respective in vitro methane emission

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Research from decades ago indicates that spent coffee grounds (SCG), a by-product rich in bioactive compounds, might mitigate methane (CH₄) emissions in ruminants, but also may impair feed intake and nutrient digestibility when included in diet. The present study aimed to investigate the effects of adding SCG at 0, 5, 10, 15, and 20%, alone or in combination with *Lactiplantibacillus plantarum* MTD/1 (LP) to fresh ryegrass before ensiling on silage fermentation quality and *in vitro* CH₄ emissions. There were 6 replicates for each of the 10 treatments. After 60 days of ensiling, silages were used for an *in vitro* ruminal fermentation experiment with the Hohenheim gas test (3 donor cows and 6 runs with 1 per treatment each). The basal diet comprised of 40% experimental grass silage, 40% maize silage, 15% grass hay, and 5% concentrate, on a dry matter (DM) basis. Data were analyzed in R using a fixed effect model for silage fermentation, with SCG and LP as fixed effect, and a mixed effect model for *in vitro* fermentation, with SCG and LP as fixed and cow and run as random effect, incorporating polynomial contrasts. After ensiling, LP the inoculation decreased silage pH ($P < 0.001$). Irrespective of the use of LP, SCG application rates linear increased ($P < 0.001$) silage DM, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber contents, while it linearly decreased ($P < 0.001$) total ash content and DM loss. Total phenols increased linearly ($P < 0.001$) in LP-inoculated silages but decreased linearly ($P < 0.001$) in those without LP. Additionally, SCG application linear enhanced silage's ferric reducing antioxidant power, DPPH radical scavenging capacity, and ABTS radical cation scavenging capacity, while LP inoculation significantly increased ABTS scavenging capacity in silage containing 10% SCG (0.00 vs. 2.87 μM Trolox/g). The *in vitro* ruminal fermentation study revealed that increasing SCG levels linear reduced ($P < 0.05$) total gas, H₂, CO₂, and CH₄ production. In addition, silages treated with LP showed lower values ($P < 0.05$) for these fermentation gas variables compared to no LP groups. The 20% SCG with LP treatment reduced CH₄ production by 11.7% compared to the 0% SCG-no LP group but concomitantly compromised *in vitro* organic matter digestibility (IVOMD, 781 vs. 729 g kg⁻¹DM). This decrease was associated with a linear decline ($P < 0.05$) in total volatile fatty acids concentration as well as acetate and propionate molar proportions (no difference for acetate molar proportion in LP-treated group). When CH₄ was normalized to IVOMD, a linear decrease ($P < 0.001$) was still observed with increasing SCG levels, with the 20% SCG-no LP and SCG-LP treatments showing 10.5 and 5.5% reductions, respectively, compared to respective controls. These findings suggest that SCG, especially in combination with LP, could be a promising silage additive for improving fermentation quality and mitigating CH₄ emissions, offering an innovative approach for integrating industrial by-products into ruminant production.

287 Assessing the perception of farmers on crop-livestock integration in Northern Nigeria: implications for animal agriculture

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Crop-livestock integration is a farming practice that combines the production of crops and livestock in a complementary manner. In northern Nigeria, a region where agriculture is crucial for livelihood, this integration has been suggested as a strategy to enhance productivity and income. However, the perceptions of farmers towards this practice remain under studied. This study assessed the perception of 200 farmer groups on crop-livestock integration across three states in northern Nigeria: Kano, Kaduna, and Katsina. A mixed-methods approach was employed, consisting of a structured survey complemented by in-depth interviews. Quantitative data were analyzed using descriptive statistics and inferential analysis. The results indicate that 75% of the respondents view crop-livestock integration positively, associating it with increased productivity and ecological sustainability. Specifically, respondents indicated an average yield per ha increase of 20% for crops and 15% for livestock in integrated systems compared to non integrated systems ($P < 0.01$). Additionally, 68% of farmers indicated that integrated farming practices significantly improved their financial resilience with up to about 30% per year. However, challenges were identified, with 62% indicating competition for land and water resources and 54% reported high costs of inputs such as fuel and feeds. Similarly, those with higher educational backgrounds were 50% more likely to adopt integrated practices ($P < 0.05$). Furthermore, exposure to extension services and loans increased positive perceptions by 40% ($P < 0.05$). The findings suggest that while crop-livestock integration has substantial potential to enhance agricultural productivity in northern Nigeria, various barriers hinder its adoption. The study underscores the need for targeted interventions including enhanced farmer education, improved access to inputs, and supportive infrastructure. This research adds to the literature on crop-livestock integration in Africa, offering valuable insights into farmer perceptions and experiences, while also calling for further investigation on the long-term impacts of such integration on rural livelihoods and animal agriculture.

139 Study of upgraded piggery biogas production and its further practical applications

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Biogas production from agricultural waste, such as piggery wastewater, represents a growing opportunity to combine sustainable energy generation with waste management. However, improving biogas quality through innovative systems remains a challenge. This study investigates the integration of a farm-scale bio-desulfurization system with a hollow fiber adsorption module (**HFAM**) to enhance biogas purification and upgrade it into bio-methane. Such systems represent cutting-edge advancements within the field, with increasing adoption globally for agricultural and industrial applications, but their optimization and scalability are still under active research. The project was conducted at the I-Yang Pig Farm, Taiwan, a commercial-scale operation with approximately 1,500 pigs producing 48 m³ of wastewater daily. Following solid-liquid separation, the wastewater containing suspended solids (**SS**), chemical oxygen demand (**COD**), and biochemical oxygen demand (**BOD**) concentrations of 176 ± 76 mg/L, 2262 ± 450 mg/L, and 846 ± 279 mg/L, respectively. Biogas derived from this wastewater was treated in a modular system combining bio-desulfurization and HFAM to remove hydrogen sulfide (**H₂S**) and carbon dioxide (**CO₂**), respectively. This modular approach is part of the broader trend toward decentralized, efficient biogas upgrading technologies. The HFAM (Aura Material Inc., Taiwan) employs advanced hollow fiber adsorption cartridges designed for high CO₂ removal efficiency. In this system, biogas first undergoes H₂S removal, achieving a 96.7% reduction after a two-month enrichment period. The desulfurized biogas is then fed into the HFAM, where CO₂ is nearly entirely removed, increasing methane concentration from 60% to approximately 94%. This upgraded bio-methane demonstrates significant potential as a sustainable energy source, with reduced CO₂ emissions compared to traditional fossil fuels. For example, replacing anthracite or cooking coal with upgraded biogas could decrease emissions by 44.4% and 42.5%, respectively. These findings highlight the novel integration of advanced bio-desulfurization and CO₂ adsorption technologies in a practical, farm-scale setup. While still emerging, the system showcases the potential for broader adoption in agriculture, contributing to carbon neutrality and sustainable energy goals. Additionally, bio-methane produced through such systems could serve as a precursor for low-carbon hydrogen production, aligning with global efforts to transition to renewable energy sources.

473 FAO LEAP guidelines on the role of livestock in circular bioeconomy systems

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Redesigning livestock systems that integrate circularity principles into livestock production represents a critical step toward a more resilient and sustainable food system. The UN Food & Agriculture Organization's (FAO) Livestock Environmental Assessment & Performance (LEAP) Partnership have published a new guideline that provides insights into the measurements and products used to strengthen circularity within the livestock sector. The guideline is designed for all stakeholders involved or associated with livestock production and explores the synergies, trade-offs, and interactions of livestock within a circular bioeconomy framework. The guideline has been developed by a dedicated Technical Advisory Group (TAG) of experts in the field on livestock production systems, nutrition and circular production systems and representing various geographical regions and interest groups. The TAG contributed to the development of methodologies, assisted in data and information collection and reviewed literature. Prior to publication, the guideline went through an extensive public review process that included academia, extension services, countries, the private sector, NGOs, global and national initiatives associated with LEAP, and other LEAP partners. Livestock play a fundamental role within a circular bioeconomy by converting non-edible biomass, such as pastures and forages, agricultural residues, and food industry by-products into high-value animal-sourced foods, organic fertilizers, and renewable energy. By recycling nutrients and utilizing low-opportunity-cost biomass, livestock contribute to reducing food-feed competition, enhancing soil health, and closing nutrient cycles, thereby improving the sustainability of agricultural systems and strengthening global food security. Beyond food production, livestock systems support bio-based industries by valorizing animal by-products such as hides, bones, and fats into materials for pharmaceuticals, cosmetics, and bioenergy. Additionally, manure-based biogas production provides a renewable energy source while mitigating greenhouse gas emissions.

The guideline presents an overview of widely used metrics and indicators for assessing the environmental impact of livestock production in a circular bioeconomy, outlining the advantages and limitations of each approach. It then examines the use of plant-based and animal-based by-products for feed, as well as the valorization of residuals, including manure, within circular bioeconomy systems. Practical examples of recovery strategies and bioeconomy innovations are provided in a regional context. Additionally, the document analyzes political and regulatory implications, discussing policies that promote the circular economy and bioeconomy, along with their strengths and challenges in supporting the use of by-products and residuals.

This document is relevant to livestock producers, supply chain partners, for example, feed producers, farming organizations, processors, and retailers seeking to enhance the environmental performance of their production systems. Non-livestock stakeholders can also benefit from its insights on creating synergies with the sector through recycling and upcycling of residuals and waste. Moreover, the guidelines are valuable for policymakers interested in fostering circular bioeconomy strategies for livestock supply chains. To facilitate user access to the guideline and support its widespread application, the guideline will be also included as part of the FAO e-learning suite and LEAP Navigator AI tool.

329 Circularity for food production with low climate and land impact

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Livestock systems provide high-quality protein for healthy diets and a growing global population. Simultaneously, they are a substantial contributor to global greenhouse gas emissions, air and water pollution and land occupation – especially ruminant systems. Contemporary research places a strong emphasis on improving current (specialised) production systems and lowering their environmental intensity per unit output (e.g. kg milk or beef), especially via improved livestock and grassland management. Implementing and measuring circularity (e.g., of nutrient flows across systems) requires a wider inter-systems perspective. The objective of this study was to investigate the impact of increased circularity of food production in Ireland via the application of a foresight approach to test more circular agri-food and land use configurations at national scale. Circular measures included use of bio-industry by-products, waste-fed insects, biorefined grass proteins, and local legume crops in livestock feed formulations, extensive use of grass-clover swards, and anaerobic digestion of manures, wastes and grass-clover feedstocks.

Nationally-available quantities of by-products suitable for animal feed, and wastes suitable for insect-farming, were collated from relevant national statistics. A linear optimisation feedstock model was developed to firstly characterise alternative feed ingredients in terms of critical properties such as digestibility, protein, fat and ash content, and then to quantify the conventional feed ingredients that could be replaced for particular livestock cohorts - prioritising imported soy and oil-palm derived products. Modified feed strategies were then integrated into the GOBLIN model. GOBLIN is a bio-physical land balancing tool that utilises extensive livestock and feed data to estimate the direct and indirect environmental and land use impacts of production systems. It is designed to explore future configurations that respect hard bio-physical constraints (e.g. land areas, soil types), but can be entirely decoupled from current economic paradigms to support genuine foresight analyses. Drawing on recent GOBLIN applications and a review of circular practices for livestock systems, scenarios were iteratively developed to combine aforementioned circular practices with the objective of achieving the following goals: (i) national feed protein self-sufficiency, (ii) climate neutrality by 2050; (iii) a halving of synthetic fertiliser-N inputs; (iv) improved resilience. In line with the foresight approach and 2050 time horizon, scenarios were considered with and without significant animal productivity improvements and ambitious agricultural mitigation technology deployment (e.g. methane and nitrification inhibitors).

Maximising the circularity of concentrate feeds via utilisation of all by-products generally resulted in lower (global) environmental impact due to a reduced need for imported feed components, but required more land nationally. This effect was particularly noticeable for confined livestock systems (pig and poultry), where circular feedstocks achieved larger reductions than many other mitigation measures. For grazing systems with limited concentrate feed inputs, mitigation measures such as methane inhibitors, clover-grass swards and anaerobic digestion had the largest mitigation effect. In all cases, increasing animal productivity and clover-grass use efficiency was critical to allow sufficient expansion of carbon dioxide removal (afforestation and bioenergy carbon capture) to achieve territorial climate neutrality as required by the Paris Agreement and national legislation.

383 DairyMix: multi-criteria assessment and decision support for sustainable circular mixed farming systems for dairy production

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Dairy production systems are an essential backbone of European agriculture but have become highly specialized, heavily relying on external inputs, lacking resilience and meeting a desirable level of circularity to a limited extent. When livestock is re-coupled with grasslands and diversified crops, dairy production systems provide valuable ecosystem services through their interaction with land, vegetation and soil. The aim of the DairyMix project (www.dairymix.eu) is to address this topic through systems thinking, defining region-specific concepts for sustainable and circular integrated crop-forestry-livestock systems for dairy production in Europe and Latin America. Data from a wide range of dairy case study farms were collected in Germany, Italy, France, Norway, Ireland, Poland, Brazil and Argentina. To assess the environmental performances of the case studies, Life Cycle Assessment (**LCA**) was carried out; economic and social sustainability indicators were also calculated. A multi-criteria assessment framework was implemented, and models covering the cropping system and other farm components were integrated to compute and assess circularity indicators. Precision farming technologies, such as the ICT-based Online Tool for monitoring Indoor barn Climate, animal stress and emission (**OTICE**), were developed, contributing to tailored solutions and management tools for regional agricultural challenges. Agroforestry practices were assessed, and qualitative in-depth interviews were conducted in selected case studies. In particular, the farmers' willingness to implement agroforestry, mixed farming and improve nutrient circularity was investigated. Preliminary findings are as follows:

- 1) Agroforestry, as a valuable multifunctional practice (e.g., increasing biodiversity, carbon sequestration, animal welfare), can provide alternative feed resources for ruminants. Nutritional value and digestibility tests on leaves of five tree species in Northern Italy revealed that mulberry (*Morus nigra* L.) has a nutritional profile comparable to lucerne or polyphytic meadows. This and other species can potentially be included as an alternative fodder in dairy cow diets.
- 2) Farmers are motivated by both environmental concerns and profitability. According to our findings, they intuitively value circular and sustainable practices and show attachment to the land, often originating from farm inheritance. Key barriers to implementing sustainable and circular practices included insufficient earnings, high workload, workforce shortages, and limited access to insurance. Bureaucracy, frequent

policy changes, and unstable expectations further hindered the adoption of such practices. Farmers notably emphasized the lack of consumer knowledge about production processes and product origins, highlighting the need for more education and awareness.

The project results are being incorporated into the DairyMix online platform. The DairyMix platform will display results from: i) the multi-criteria assessment, allowing the users to evaluate the effect of varying the weights of the sustainability principle considered; ii) the modelling of mitigation measures and alternative cropping scenarios in representative dairy production systems across Europe and Latin America. Users will be asked for feedback, which will be incorporated into the platform. In contrast to “one-fit-all” solutions, the DairyMix interactive platform will present a range of options for the sustainability of farming systems for dairy production, favouring the adoption of informed decisions for circular and integrated crop-forestry-livestock in different regions.

113 Circular economy in animal agriculture among Africa's Maasai people: a sustainable model for resilient livelihoods

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The Maasai people of East Africa, renowned for their pastoralist lifestyle, provide a compelling example of a circular economy approach to animal agriculture. This paper examines how the Maasai have integrated traditional knowledge, sustainable practices, and cultural values to create an efficient, low-waste agricultural system that supports their communities while maintaining environmental resilience. By leveraging the principles of resource optimization, waste minimization, and regeneration, the Maasai demonstrate a unique application of circular economy principles in animal agriculture. The Maasai's economy is built around livestock, primarily cattle, goats, and sheep, which provide milk, meat, hides, and other resources. Central to their circular model is the utilization of every part of the animal, ensuring minimal waste. For instance, animal hides are used to make clothing, bedding, and household items, while bones and horns serve as tools or ornaments. Additionally, manure is employed as a natural fertilizer to enhance soil fertility, supporting small-scale crop cultivation and promoting regenerative agriculture. This integration of livestock and crop farming ensures a self-sustaining system that maximizes the utility of available resources.

Water scarcity and land degradation, critical challenges in the arid and semi-arid regions inhabited by the Maasai, have prompted innovative adaptive practices. The Maasai have developed rotational grazing systems, which allow vegetation to recover and prevent overgrazing. These systems align with circular economy principles by maintaining ecosystem balance and reducing the risk of soil erosion and desertification. Furthermore, the Maasai's reliance on indigenous knowledge of seasonal changes and resource availability ensures efficient allocation of grazing land and water resources.

Social cohesion and communal sharing are integral to the Maasai's circular approach. Traditional practices, such as cattle lending and reciprocal herding arrangements, enable equitable resource distribution while fostering resilience during droughts or economic hardship. These practices emphasize the importance of collective action in sustaining both livelihoods and the environment. However, the Maasai's circular economy model faces increasing pressures from climate change, population growth, and encroachment by modern agricultural systems. These challenges threaten the delicate balance of their resource-efficient practices. To address these issues, there is a need for targeted interventions that combine traditional knowledge with modern technologies. For example, the introduction of renewable energy solutions, such as biogas digesters using animal waste, can enhance energy access while reducing environmental impact. Similarly, value-added processing of livestock products, supported by market access initiatives, can improve economic returns without compromising sustainability.

This study highlights the Maasai's circular economy practices as a source of inspiration for sustainable animal agriculture in Africa and beyond. Their system demonstrates the potential of traditional knowledge and cultural values in shaping resilient, environmentally sound livelihoods. By supporting the Maasai through policies that protect their land rights, promote sustainable grazing practices, and facilitate access to climate-resilient technologies, their circular economy model can be strengthened and scaled to address broader challenges in agricultural sustainability.

207 Household Biogas Adoption in Kericho, Kenya: Barriers & Drivers for Sustainable Energy

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Agriculture is a major contributor to global greenhouse gas emissions but also holds significant potential for climate change mitigation through the adoption of circular economy principles. Circular economy strategies, such as nutrient recycling, composting agricultural waste, and integrating renewable energy, offer a way to reduce emissions and enhance sustainability in agricultural systems.

This paper examines the role of circular economy practices in reducing agricultural emissions, with a focus on ecosystem restoration efforts in Kenya. Drawing on case studies from the Hearts of Green Organization, it highlights community-led initiatives like tree planting, forest adoption, and agroforestry, which serve as practical examples of how circular economy principles can be applied. These activities contribute to carbon sequestration, soil restoration, and enhanced biodiversity, while reducing reliance on synthetic fertilizers and improving long-term farm sustainability.

In addition to the practical applications of circular economy principles, the study explores the policy frameworks and governance structures necessary to scale up these initiatives. It emphasizes the importance of policies that incentivize farmers to adopt sustainable practices, such as subsidies for green technologies and support for local agroforestry projects. Public-private partnerships and community engagement are identified as key factors for ensuring the success and scalability of circular economy models in agriculture.

By closing resource loops, circular economy principles can help transform agriculture from a high-emissions sector into a sustainable, climate-resilient system. The study concludes that circular models can mitigate greenhouse gas emissions, improve soil health, promote biodiversity, and offer economic benefits to farmers.

The research demonstrates the transformative potential of circular economy practices in agricultural systems. These models not only address the urgent need to reduce agricultural emissions but also provide a pathway for farmers and communities to adapt to climate change. Through ecosystem restoration, resource efficiency, and sustainable practices, circular economy principles can contribute to the global effort to combat climate change while fostering long-term agricultural sustainability.

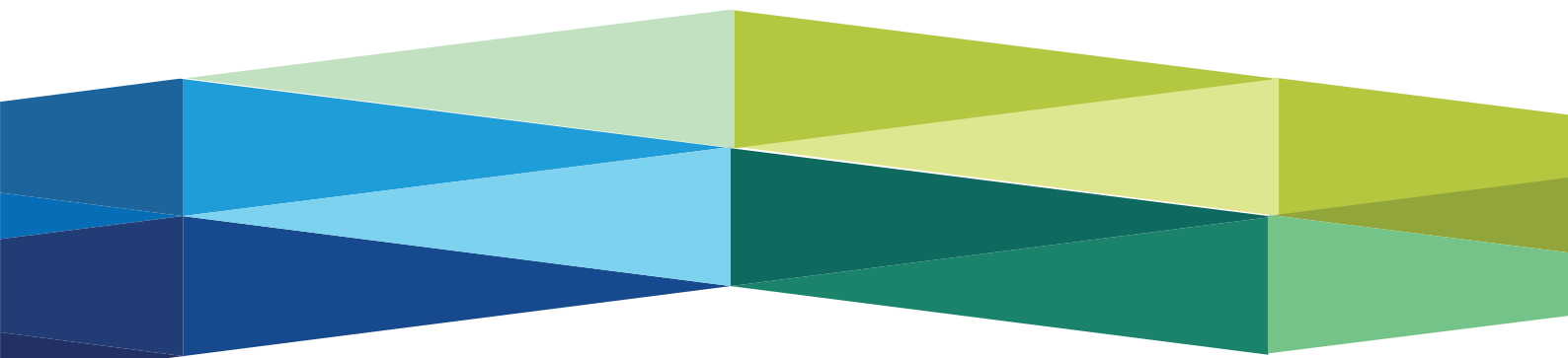


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Theme 7: Scaling and modelling of greenhouse gas emissions

7.1 Modelling and mitigating enteric methane emissions in ruminants – Perspective on diet, management, and technological interventions



210 Estimating enteric methane emissions in South Asian countries using feeding trial data in different models

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Emitted methane from cattle significantly contribute to greenhouse gas emissions in South Asia, particularly in India, Bangladesh, and Pakistan. These countries are home to approximately 250 million lactating and non-lactating cattle, collectively producing around 300 million tons of milk annually. While direct measurement of enteric methane (**EntCH₄**) emissions through calorimetry is precise, it is resource-intensive and impractical for large-scale implementation. Mathematical models, widely recommended by the Intergovernmental Panel on Climate Change (**IPCC**), offer a practical alternative for estimating EntCH₄ emissions by utilizing feeding trial data and accounting for dietary intake and feeding characteristics. In this context, this study analyzed EntCH₄ emission estimates generated by six widely used models tailored for global and tropical livestock systems. These models are categorized based on dry matter intake (**DMI**) and gross energy intake (**GEI**) and include: IPCC_{DMI} and IPCC_{GEI} (<http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.html>), Ribeiro_{DMI} and Ribeiro_{GEI} (doi:10.1017/S1751731120001743), and Patra_{DMI} and Patra_{GEI} (doi:10.1007/s11027-015-9691-7). Feeding trial data from 91 studies were used to estimate EntCH₄ emissions for lactating and non-lactating (dry cow, bull, heifers) cattle across India (59 trials: lactating cattle = 35, non-lactating cattle = 24), Bangladesh (20 trials: lactating cattle = 13, non-lactating cattle = 7), and Pakistan (12 trials: lactating cattle = 12). The dataset included 1,684 cattle representing crossbred (1,193), Sahiwal (173), Indigenous (279), and Holstein Friesian (39) breeds, with an average tropical livestock unit (**TLU**) of 1.40±0.42 (1 TLU = 250 kg live body weight), distributed as crossbred (1.42±0.30), Sahiwal (1.76±0.52), Indigenous (1.01±0.38), and Holstein Friesian (1.69±0.18). Mean DMI, GEI, and milk yield were 9.43±4.11 kg/day, 109.75±50.86 Mcal/day, and 9.43±4.11 kg/day, respectively. For lactating cattle, the IPCC_{DMI} model estimated significantly ($P < 0.05$) higher EntCH₄ emissions (243 g/day/cattle) compared to Ribeiro_{DMI} (205 g/day/cattle) and Patra_{DMI} (202 g/day/cattle). Non-lactating cattle followed a similar pattern, where IPCC_{DMI} model yielded 124 g/day/cattle EntCH₄ which was 10g higher than both of the Ribeiro_{DMI} and Patra_{DMI} models. GEI-based models for lactating cattle also showed higher emissions (g/day/cattle) with IPCC_{GEI} (191) which was 22 and 24 g higher than the Ribeiro_{GEI} and Patra_{GEI} models, respectively. On the other hand, non-lactating cattle emits similarly while GEI was in consideration which ranged from 99 to 102 g/day/cattle. Across countries, no significant differences ($P > 0.05$) in EntCH₄ emissions (g/day/TLU) were observed for lactating or non-lactating cattle across most of the models, except Ribeiro_{DMI} (India = 106 g/day/TLU vs. Bangladesh = 124 g/day/TLU) and Patra_{DMI} (India = 105 g/day/TLU vs. Bangladesh = 122 g/day/TLU) in non-lactating cattle ($P < 0.05$). These findings reveal that IPCC models tend to overestimate EntCH₄ emissions and highlight the variability in emission estimates across models. This underscores the critical need to select context-specific models for accurate regional assessments and to refine existing methodologies or developed new models by using feeding trial/animal data to enhance their applicability to diverse production systems in South Asia.

306 Carbon footprint in different tropical dairy farms: the role of metrics

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The livestock sector is confronted with numerous challenges, including the imperative to enhance the production of animal-based foods to feed a growing global population, while simultaneously adapting to climate and economic changes and improving animal performance within more sustainable production systems. In 2023, the dairy herd contributed only 10% of the GHG emissions from Brazilian livestock. The aim of this study was to assess the carbon balance of different tropical production systems, namely pasture-based, partial confinement, and confined systems. Data was collected through questionnaires administered on visits to 40 dairy farms located in the states of São Paulo, Minas Gerais, Goiás and Rondônia. Farms were visited in 2023 to conduct interviews, which lasted an average of three to four hours. A structured questionnaire was administered to collect information on dairy production systems related to the production unit, dairy herd, total diet composition, manure management, feed production and acquisition, land use change, and tree planting. All data collected referred to the production process in the year before. Dairy farms were classified into three groups based on their production system: pasture-based farms (n=16), where animals have free access to pasture and a significant portion of their diet comes from grazing, partial confinement (n=10), where animals have access to pasture for part of the day, but receive additional feed supplementation, usually in bunks, to complement their total diet and confined farms (n=14), where dairy cattle are kept in enclosed environments year-round and receive their entire diet from the feed bunk. To calculate the carbon footprint, in CO₂eq per kilogram of milk, the Embrapa Low Carbon Livestock Calculator was used, based on the IPCC Tier 2 model (IPCC, 2019), which follows the FAO/LEAP guidelines (FAO, 2016), meets the requirements of ISO 14.044 (ISO, 2006) and the ICVCalc food library (Matsuura et al., 2022). The variables of interest followed a normal distribution (Shapiro-Wilk test). The data were submitted to analysis of variance and comparison of means by the Fisher test (5%), using the command PROC MIXED of SAS® 9.4. The pasture-based systems exhibited the highest carbon footprint (2.528 kg CO₂eq⁻¹ kg FPCM), significantly differing (P < .0001) from the partial confinement (1.715 kg CO₂eq⁻¹ kg FPCM) and confined (1.244 kg CO₂eq⁻¹ kg FPCM) systems. No significant difference was found between partial confinement and confined systems. This study found that partial confinement and confined systems exhibited lower carbon footprint compared to pasture-based systems. These results emphasize the need for targeted strategies to optimize production efficiency and reduce the carbon footprint of various dairy production systems, but must be analyzed in conjunction with other metrics related to the economic and social performance of properties, at the risk of excluding small family pasture producers from global carbon financing incentives.

378 The potential reduction in New Zealand's agricultural emissions arising from the adoption of new technologies

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Emissions from the New Zealand agricultural sector comprise approximately 50% of reported national anthropogenic greenhouse gas emissions. A substantial drop in these emissions is needed to meet domestic and international reductions targets and New Zealand is investing heavily in the development and commercialisation of agricultural emissions reduction technologies and practices.

This paper outlines the results of a modelling approach estimating the potential reduction in agricultural non-CO₂ emissions that could be achieved out to 2050 by the development, commercialisation and adoption of mitigation measures by New Zealand farmers. These measures include those currently under development in New Zealand plus additional interventions that are relevant to New Zealand farm systems and close to commercialisation in New Zealand.

Reductions are calculated relative to an existing baseline emissions projection. Timing of availability, efficacy and cost for each mitigation measure are based on expert judgement using best available information. Adoption rates are modelled using the CSIRO ADOPT model with inputs from selected New Zealand stakeholders and experts (farmers, extension specialists, researchers, and representatives from sector good bodies). Key assumptions informing those inputs, consistent with the baseline, include an emissions price incentive for adoption of mitigation measures of at least \$100/tCO₂e avoided as well as qualitative, non-price incentives arising from freshwater policy, forestry, and international supply chains.

Greenhouse gas emission reductions are estimated for each mitigation measure individually and for the full suite of interventions by sector (dairy, beef, sheep, deer) and for agriculture as a whole.

Based on the assumptions regarding timing, efficacy and adoption, we estimate that the suite of mitigation interventions assessed could achieve a reduction in total agricultural non-CO₂ emissions of 29% by 2050 relative to 2017, and a reduction in biogenic CH₄ emissions from agriculture of 32% by 2050 relative to 2017.

107 Predicting methane emissions of feedlot beef cattle fed white-grain based diets

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Most modelling studies of methane (**CH₄**) emissions from grain-fed cattle draw heavily on corn-based diets, and specific corn-based equations are available to use in national inventories. This study aimed to evaluate the performance (i.e., precision and accuracy) of existing prediction models for CH₄ emissions from beef cattle fed barley-based feedlot diets and to develop improved equations tailored to such production systems. A database was assembled of 713 individual open-circuit respiration chamber CH₄ measurements of 175 animals from 12 studies of beef cattle of various breeds and breed-types, fed a range of feedlot diets based on barley and wheat and containing 25 ppm monensin, as is typical of Australian feedlot diets. The database also included a wide range of factors that are known to impact CH₄ production such as dry matter intake (**DMI**), ether extract (**EE**), crude protein, cell components, energy and liveweight.

Eight published equations for prediction of CH₄ emissions from cattle fed feedlot diets were evaluated using a subset of 384 observations from the database (mean $\pm$ s.d. = 79.9 $\pm$ 27.5 g CH₄/d) derived from Angus cattle fed barley-based diets. All evaluated equations lacked accuracy and precision in predicting CH₄ emissions for the diets fed in this study (RMSE ranged from 39.3 – 119 g CH₄/d). Average mean bias for extant equations was 69.8 g/d (ranging from 17.8 – 115 g CH₄/d) and all equations displayed significant linear bias.

Given these findings, two specific equations were developed using this database sub-set, a CH₄ yield equation based on DMI: CH₄ g/d = 9.89 $\pm$ 1.54 $\times$ DMI (n = 384; *P* < 0.01; RMSE = 32.6 g/d; *r*² = 0.85); and an equation based on DMI, neutral detergent fibre (**NDF**) and EE: CH₄ (g/d) = 5.11 $\pm$ 1.58 $\times$ DMI - 4.00 $\pm$ 0.821 $\times$ EE + 2.26 $\pm$ 0.125 $\times$ NDF (n = 384; *P* < 0.05; RMSE = 22.2 g/d; *r*² = 0.91).

Validation was performed with an independent data subset (remainder 329 observations, mean $\pm$ s.d. = 86.9 $\pm$ 31.2 g CH₄/d) gathered after the development dataset, and representing a broader range of cattle breeds and dietary compositions (including both wheat- and barley-based diets) so as to test the applicability of the equations to variation encountered in industry. The first proposed equation (based on DMI only) overestimated methane production by 10.9 $\pm$ 1.59 g/d (*P* < 0.01; RMSE = 33.1 g CH₄/d), but had significant linear bias (-0.509 $\pm$ 0.0893; *P* < 0.01). The proposed DMI+NDF+EE model outperformed existing corn-based equations and the DMI-only model: showing a mean bias of -5.42 $\pm$ 1.51 g CH₄/d (*P* < 0.01; overestimation, but the smallest among all tested equations), with no linear bias (-0.0136 $\pm$ 0.113 g CH₄/d; *P* = 0.90) and better fit (RMSE = 28.8 g CH₄/d) than any of the literature equations.

This study underscores the importance of incorporating dietary factors into methane prediction equations for feedlot cattle. While corn-based models demonstrate partial applicability, region-specific equations tailored to barley- and wheat-based diets offer improved accuracy and reliability for such diets, particularly given the inherent variability of enteric CH₄ emissions.

275 Methane emission estimation of dairy cows in Latin-American database using machine learning tools

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Several mathematical models to predict individual methane emission have been based on multiple linear regressions (**MLR**) using as predictor variables dry matter intake (**DMI**), diet composition and milk yield. However, the use of algorithms and machine learning (**ML**) tools have been reported only for European countries but not in tropical countries. The aim of this study was to evaluate performance of ML in enteric methane prediction (**eCH₄p**) emissions from dairy systems. Thirty-one combinations of eleven variables for forage quality, and animal features were defined to predict the methane emissions for dairy cattle from studies conducted in the Latin America and Caribbean (**LAC**) region (Congio et al., 2021). Exploratory analyses were used on the data and were defined as a subset of 588 records. The data was divided into the train (80%) and test data set to evaluate the predictive machines, these used multiple regression with ordinary least squares (**MLR**), least absolute shrinkage and selection operator (**LR**), ridge regression (**RR**) and random forest (**RF**). The machines were compared using the five cross-validation method with the values of Predictive Ability (**PA**), Mean Absolute Error (**MAE**), Root of Mean Square Prediction Error (**RMSPE**), and Predictive Correlation (**PC**). The models were ranked and selected those with smaller values for PA and RMSPE. The RStudio 4.3.3 was used to develop the analysis. The mean value for methane emissions was 316.6 (± 90.5 grams per day). The RF with full input variables had the best predictive performance with PA value of 0.15 and a predictive correlation of 85%. The best predictive machine with the least input variables was for Total DMI and neutral detergent fiber with the RF, it is remarkable that nearly 96 different variables and method combination got PA lower than 0.2 and PC over 70%. The combinations of input variables to know the eCH₄p, suggest that is necessary strengthen the measurements in dairy farms to enhance the training process of predictive machines with best fit and accuracy of ML for predictive modelling.

294 Implications of farm adaptation to a changing climate on greenhouse gas emissions of livestock production

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The climate across eastern Australia has changed over recent decades, with increasing temperatures and changing rainfall patterns across the region, requiring changes on livestock production systems. These systems are also under pressure to reduce greenhouse gas (**GHG**) emissions. This study investigated the effect of changes to livestock farm production systems designed to adapt to a changing climate on whole farm GHG emissions and emissions intensity (**EI**).

The case study farm business was in East Gippsland, Victoria, Australia (-37.50, 147.84). The grazable area of the farm was 600 ha (40 ha annual forage cropping, 181 ha sown temperate pastures and 379 ha of unimproved pasture). Livestock consisted of a 1200 self-replacing composite ewe flock, 250 cow self-replacing beef cattle herd and traded livestock depending on seasonal conditions. The region has a temperate climate with an annual average rainfall of 670 mm. Two farm options were compared: (1) Base, which operated the business as it is currently; and (2) Adapt, which used currently available technologies to improve pasture and animal production to suit the changing climate. The changes in the Adapt option were defined by a regional reference group of farmers and were considered achievable targets for increasing farm performance. The changes were to increased feed supply (by sowing more improved pastures and reducing paddock size to improve pasture utilisation), increased animal performance through improved genetics (lambing increased from 150 to 165%, heifer calving rate increased by 5% and calf growth rate increased by 5%) and reducing emissions (earlier sale of lambs and tree planting on 12 ha of farm land).

Pasture growth was modelled using the SGS Pasture model to predict the monthly pattern of pasture growth using climate data from 2000-2019. This was used to calculate livestock production and supplementary feed requirements in a spreadsheet-based feed demand and supply calculator. The SB-GAF calculator was used to estimate greenhouse emissions using the Australian government approach. Total farm emissions were expressed as tonnes of carbon dioxide equivalents (**t CO₂e**) per year. The EI was estimated for beef, sheep meat and wool based on the amount of product sold. The protein allocation method was used to partition sheep GHG emissions to meat and wool.

Total annual net farm GHG emissions were 2701 t CO₂e in the Base farm, and this increased to 2731 t CO₂e in the Adapt farm. The EI of sheep meat, greasy wool and beef production on the based farm were 6.1, 22.6 and 12.0 t CO₂e/t product sold, with corresponding values of 5.6, 20.7 and 10.3 in the Adapt farm.

The Adapt option increased net GHG farm emissions but reduced EI of sheep meat, wool and beef production. The increased reproductive and growth rates resulted in a higher number of animals (increasing net farm GHG emissions), but there was also more product sold (reducing the EI). The results highlight the synergy between climate adaptation and reducing EI, but further research is needed to find practical options to reduce net farm GHG emissions on pasture-based livestock farms.

98 Supporting the transition towards carbon-zero dairy systems: the New Zealand case-study of the ClieNFarms programme

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New Zealand (NZ) agriculture contributes almost half of the nation's greenhouse gas (GHG) emissions, with ~70% and ~20% of these agricultural emissions deriving from enteric methane from ruminants and nitrous oxide from urine patches, respectively. Under international and domestic GHG agreements, NZ is committed to reduce its enteric methane emissions by 24-47% and achieve net zero emissions of all other greenhouse gases by 2050. The NZ government has indicated that by 2030, agricultural emissions in NZ will be priced, and farmers will have to start paying for the GHG emissions from their farms. To help support the transition of NZ dairy farms towards reduced GHG emissions and net carbon zero, NZ is partnering in the international ClieNFarms project. ClieNFarms is a Horizon 2020 project funded by the European Commission and aims to co-develop locally relevant solutions to foster climate-resilient farming. The Southern Dairy Hub is the NZ case study farm. This farm has four research farmlets that were used to examine the effects of 'system intensity' (standard vs low intensity; 180 kg N/ha fertiliser applied and 3 cows/ha vs 50 kg N/ha and 2.5 cows/ha) and 'wintering system' (crop- vs pasture-based wintering) factors on farm system production, GHG emissions, nitrate leaching losses and farm operating profit. Data collected from each farmlet included milk production, number of animals, stocking rates, N fertiliser used, pasture DM grown, and imported feed. GHG emissions were estimated based on these measurements, using the NZ farm systems tool FARMAX. Emissions per ha and per unit of milk were 20% and 7% lower, respectively, for the 'low intensity' systems compared with the 'standard intensity' systems. 'Wintering system' did not affect emissions per ha, but emission intensities were ca. 4% higher for 'pasture wintering' compared with 'crop wintering'. The research farmlets were then also augmented with future scenario modelling, again using the FARMAX model, to assess the impact of combinations of currently available mitigation and management options on GHG emissions and abatement costs. These options included: buying the carbon credits to offset GHG emissions, using animals with higher genetic merit, using alternative sward species or home-grown crops, using lower footprint bought-in feed, eliminating N fertiliser inputs, in-setting production trees, and converting part of the dairy farm to grow alternative cash crops or trees. Modelling indicates that with currently available options, on-farm GHG emissions could be reduced by up to 30% for the standard intensity systems. The 'No N fertiliser' option resulted in the largest GHG reduction, especially when it was combined with in-setting trees. Overall, the analysis demonstrates that while effective mitigation options exist for intensive pasture-based dairy production, these generally incur a production and financial cost. Further, these losses are higher for more-intensive systems, reflecting that pricing and policy need careful attention if their costs are to be correctly ascertained. Further investment in cost effective tools and techniques to reduce the environmental footprint of grazed dairy production remains an international priority.

296 Evaluation of partial least squares regression as empirical model for enteric methane in dairy cows

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Linear regression with multiple predictors is often used for empirical models that predicts methane yield (g/kg dry matter intake). The current model used in Denmark for national greenhouse gas inventories for dairy cows includes dry matter intake and dietary concentration of five components as predictors: ash, crude fat, crude protein, neutral detergent fiber, and starch. These predictors can be correlated, resulting in collinearity. This study tested whether partial least squares regression can produce more robust slope coefficients for the prediction of enteric methane production and yield in dairy cows, by accounting for the collinearity between the predictors. Furthermore, model accuracy was tested with and without the inclusion of the squared dry matter intake as predictor. A dataset from 24 trials including data on dry matter intake and methane production from lactating dairy cows and dairy heifers in respiration chambers, with a total of 554 observations was compiled. The Danish prediction model was fitted either in a linear regression or in a partial least squares regression, and in both cases with and without the addition of the squared dry matter intake. In the partial least squares regression, the first five principal components were used, as using all components would result in identical coefficients as linear regression. The coefficients for each model were estimated 300 times, each time randomly selecting 70% of data within each individual trial and using the remaining 30% of the data to test the model and calculating the root mean square error of the predictions. Anova and Tukey test were used to compare the models. The lowest root mean square error ($P < 0.001$) for methane yield was found in the linear regression with squared dry matter intake (2.28 ± 0.112 g CH₄/kg dry matter intake), followed by the linear regression without squared dry matter intake (2.43 ± 0.113 g/kg), the partial least squares regression with squared dry matter intake (2.49 ± 0.116 g CH₄/kg dry matter intake), and finally the partial least squares regression without squared dry matter intake (2.58 ± 0.119 g CH₄/kg dry matter intake). The coefficients for the linear regression model without squared dry matter intake were 32.7 ± 1.28 g CH₄/kg dry matter intake for the intercept, -0.192 ± 0.0171 for dry matter intake (kg/d), and -0.0341 ± 0.0057 , -0.0591 ± 0.0059 , -0.00116 ± 0.0019 , -0.0127 ± 0.0015 , -0.0097 ± 0.0031 for the concentration of ash, crude fat, neutral detergent fiber, starch, and crude protein (g/kg dry matter), respectively. The coefficients for the partial least squares regression without squared dry matter intake were 32.8 ± 1.27 for the intercept (g CH₄/kg dry matter intake), -0.145 ± 0.0211 for dry matter intake (kg/d), and -0.0555 ± 0.0058 , -0.0580 ± 0.0067 , -0.0000184 ± 0.0019 , -0.0173 ± 0.0016 , -0.0047 ± 0.0031 for concentration of ash, crude fat, neutral detergent fiber, starch, and crude protein (g/kg dry matter), respectively. With our data, linear regression was more accurate than partial least squares regression, with the foremost having a higher weight the dry matter intake coefficient than the latter.

310 Interactions between pasture biodiversity, milk production and greenhouse gas emissions in small African dairy systems

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The highlands of Kenya comprise some of the most biodiverse regions of the world. In Kenya, tropical highland regions account for only 11% of the total land area, but are home to the majority of the country's population. These regions, however, are under significant stress as a result of human-induced climate change and land-use change. Dominating the landscape in densely populated areas of the East African highlands are smallholder livestock farms practicing extensive and semi-intensive farming, often relying on semi-natural grasslands and managed pastures for feeding their livestock. These farms are critical to the ecological dynamics of these ecosystems. Smallholder farms account for 80% of all farms in Kenya (and pasture accounts from 53% to 100% of feed dry matter intake by cattle, depending on season). Despite the significant research focus on livestock-rangeland dynamics in Kenya, the interplay between biodiversity, livestock and pastures on smallholder farms in the highlands remains largely unexplored. This research gap necessitates a thorough investigation of the ecological and economic implications of pasture management in these biodiverse and vulnerable areas. Understanding these issues would help guide policy for increasing milk production meeting future demand. Anticipated population and income growth in African countries is predicted to significantly increase the demand for dairy products in the coming decades. According to the United Nation's Food and Agriculture Organisation, Africa's demand for meat and milk is expected to increase between by up to 400% by 2050 compared to 2010 levels. Kenya reflects these continental-wide trends with demand for milk set to increase by 38% by 2050 compared to levels in 2019. To meet this increasing demand, the Kenyan government has pledged to increasing its production by 150% by 2030. To study the interaction between pasture diversity, milk production and greenhouse gas emissions we surveyed 60 farms in Bomet County Kenya with a relatively large amount of pasture, selected from a wider panel of representative farms. The farms were divided into two groups of 30 pioneer farms and 30 control farms. Pioneer farms were those with higher milk productivity per animal, higher adoption of innovative practices and with proxy variables (e.g. low herbicide use) likely to indicate higher pasture biodiversity. Statistical matching was used to select control farms that matched the pioneers on the basis of pasture amount, altitude, number of lactating dairy cows and soil colour. For all 60 farms soil properties and pasture biodiversity (species number and Shannon diversity) were measured. A detailed on-farm survey was carried out of feed properties and dairy system production characteristics. Using this information a greenhouse gas footprint was estimated for each farm (kg CO₂ equivalent per L milk). Here we report the relationship between these variables which give insights into the role of pasture diversity.

409 Enteric methane factors for cattle production systems in Kenya

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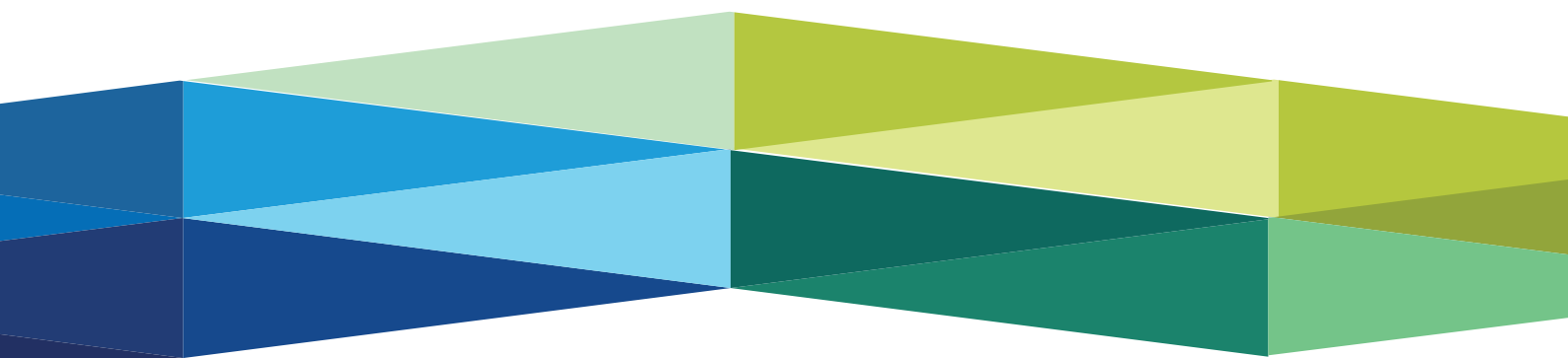
In Kenya the agricultural sector significantly contributes to greenhouse gas (**GHG**) emissions, with livestock production being a notable source of methane. The objective of this study was to evaluate the enteric methane emissions across three cattle production systems in South Eastern Kenya: extensive, semi-intensive, and ranching. Data were collected from 185 farms using the Rural Household Multi-Indicator Survey (**RHoMIS**) tool. Collected data were supplemented by literature to address data gaps. Data was collected by season to capture the seasonal effect on annual emissions. Data analysis followed the Intergovernmental Panel on Climate Change (**IPCC**) Tier 2 methodology published in 2019. Emission factors were derived from the gross energy intake, which was estimated based on the animals' net energy requirements and the digestibility of their feed. Calculations were performed for the following cattle categories: cows (>36 months), bulls (>36 months), juvenile males (12-36 months) and juvenile females (12-36 months). Data analysis included descriptive statistics (mean, standard deviation, median, interquartile range) and non-parametric tests (Chi-square, Kruskal-Wallis), with post-hoc Dunn tests for significant differences between cattle production systems. Results revealed significant differences in emissions factors between production systems, with ranching consistently exhibiting the highest emission factors. For cows, annual emission factors (Kg eCH₄ head⁻¹) ranged from 51.0 ± 13.4 (semi-intensive) to 72.5 ± 12.8 (ranching). Bulls showed similar trends, with annual values ranging from 46.6 ± 6.8 (semi-intensive) to 75.4 ± 6.9 (ranching). Juvenile females had lower emissions, with annual factors between 35.6 ± 4.2 (extensive) and 46.6 ± 3.7 (ranching). Juvenile males exhibited the lowest emission factors, ranging from 32.5 ± 4.2 in semi-intensive systems to 45.4 ± 3.8 in ranching systems, except in extensive systems. Overall, all cattle production systems exhibited low and similar digestibility values for the feed basket, ranging from 57.4% to 58.5% during the dry season and from 57.6% to 58.0% during the wet season. These insights are valuable for informing future updates to greenhouse gas inventories and guiding the development of climate-resilient livestock management practices that account for the characteristics of each production system. Further, they show that by focusing on tailored feed management strategies and improving feed digestibility, it might be possible to reduce methane emissions while maintaining or enhancing protein output.



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7.2 Scaling and modelling of greenhouse gas emissions



282 Assessing mitigation strategies for enteric methane emissions in Sub-Saharan Africa's ruminant production systems

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Livestock production in sub-Saharan Africa (**SSA**) contribute significantly to national greenhouse gas (**GHG**) emissions, particularly enteric methane (**eCH₄**) and nitrous oxide (**N₂O**) from enteric fermentation and manure management, respectively. Various enteric CH₄ mitigation strategies have been implemented to reduce the environmental impact of livestock production in this region and reported in the literature. This research focused on aggregating eCH₄ data from limited *in vivo* studies in SSA to explore emissions variability and identify specific greenhouse gas mitigation strategies for the region. PRISMA scheme which consists of studies' eligibility, screening, selection, and inclusion steps was used on *in vivo* data of GHG emissions from ruminants in SSA. These results contributed to a web-based application (Metheix) that standardizes data, automate constraint checks, and enable scalable access, visualization and statistical analysis (removal of outliers, modelling, evaluation) of studies on eCH₄ mitigation strategies in SSA for different categories of ruminants (cattle, sheep, goats). A total of 21 publications were identified, comprising; 14 studies in South Africa, 5 in Kenya and 2 in Burkina Faso. Among these, 13 publications focused on *in vivo* eCH₄ emissions data from cattle, 5 from sheep and 3 from goats. Data on publication details, trial conditions, animal characteristics, diets' chemical composition, digestibility parameters, eCH₄ emissions, excretion and performance variables were compiled, resulting in a dataset containing 84 records of treatment means. The studies were categorized based on the mitigation strategies investigated, mostly dietary strategies. Specifically, 9 studies investigated the impact of plant secondary metabolites, including tannins and other bioactive compounds, on eCH₄ emissions. Additionally, 3 studies examined the effect of improved forage quality, while 6 studies focused on inclusion of concentrates. Lastly, 3 studies explored the influence of feeding levels adjustments and considered variations linked to age and breed. Descriptive analysis of our dataset indicated that average eCH₄ yield (CH₄ kg DMI⁻¹) obtained for dairy cows were in average 23.2 g CH₄ kg DMI⁻¹, with values ranging from 16.9 and 29.1 g CH₄ kg DMI⁻¹. Other mature cattle showed an average methane yield of 24.0 g CH₄ kg DMI⁻¹, ranging from 21.1 to 27.7 g CH₄ kg DMI⁻¹, while growing cattle had average values of 27.8 g CH₄ kg DMI⁻¹, with a range of 18.7 to 34.5 g CH₄ kg DMI⁻¹. For sheep, the average methane yield was 21.9 g CH₄ kg DMI⁻¹, with values between 16.8 and 26.5 g CH₄ kg DMI⁻¹. Whereas, goats showed the highest variability when compared to other animal categories, with an average yield of 31.7 g CH₄ kg DMI⁻¹ and values ranging from 12.6 to 49.8 g CH₄ kg DMI⁻¹. This dataset provides additional valuable baseline for assessing methane emissions in SSA's livestock systems and informs the development of specific mitigation strategies. Validation of existing eCH₄ models or developing new Tier 2 models, that are suited for specific conditions of SSA's livestock systems are on-going.

215 Modelling GHG emissions from livestock using GLEAM: a sensitivity analysis of parameters and emission factors

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Global Livestock Environmental Assessment Model (**GLEAM**), a spatially explicated model developed by the Food and Agriculture Organization of the United Nations (**FAO**), uses georeferenced livestock data to estimate the environmental impact of the livestock sector. It applies a life cycle assessment approach to calculate emissions throughout the entire production chain. However, data availability is limited in many regions, and its collection requires significant effort and is subject to inherent uncertainty. Evaluating the impact of input parameters, a process not yet explored in detail for GLEAM, is essential to optimize data collection and reduce error propagation in greenhouse gas estimates. Sensitivity analysis has emerged as a key method for evaluating the influence of individual parameters, helping identify the most influential ones, facilitating model refinement and prioritization efforts.

This study applied sensitivity analysis to input parameters in GLEAM, including animal parameters and emission factors. Global data from 16 large ruminant production systems, incorporated in GLEAM's global estimates, were analyzed and used for this study. To efficiently process over 5 million records, sample datasets preserving spatial variability were created. To do this, parameters were categorized as: 1) those acting as conditions within the model, retaining all spatial data, and 2) for the rest, a fixed value was assigned.

A comprehensive analysis of the processes involved in animal emission estimation was conducted to evaluate the completeness of data for each parameter. Parameters with known variability were treated as global parameters, using their full data range in the sensitivity analysis. For others, a fixed range of $\pm 20\%$ was applied. The sensitivity analysis varied one parameter at a time while holding all others constant. The analysis predicted the influence of 70 out of 96 parameters ($R^2 > 0.70$). For these, a sensitivity index was calculated, and their correlations with methane and nitrous oxide emissions were determined. Parameters were ranked based on the absolute value of their sensitivity index: high (index > 1), moderate (0.2–1), low (0.10–0.2), and extremely low (< 0.1).

The results identified the most influential parameters, with diet digestibility standing out due to its negative correlation with all types of emissions and an absolute sensitivity index at least twice as high as any other parameter. Additionally, the analysis identified 21 parameters with moderate to high sensitivity: four for methane emissions from enteric fermentation, seven to ten for methane emissions from manure, and six to ten for nitrous oxide emissions from manure. In contrast, nearly 49 parameters were classified as having low to extremely low sensitivity across all direct emissions.

This study offers valuable insights for prioritizing data collection efforts, improving data quality for the most influential parameters, and reducing uncertainty in greenhouse gas estimates. It also provides guidance for scenario simulations, predicting the impact of mitigation strategies on emissions. The results from the sensitivity analysis will be used to identify key parameters, whose uncertainty will be collected and used in simulations to estimate uncertainty of global estimates in GLEAM.

343 Economic-climatic synergies and trade-offs in cattle production

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Improving economic performance and reducing greenhouse gas emissions are key challenges for the cattle farming industry. Decisions regarding landuse, feeding strategies, and slaughter practices directly affect both economic results and emissions at the farm level. Often, optimizing these decisions economically also lowers emission intensity. The potential for such win-wins is well-known and includes measures like improving forage quality, extending the productive life of dairy cows, increasing milk yield, reducing the age at first calving, and intensifying beef production from bulls. However, knowledge about the circumstances under which these decisions produce win-wins, and when they might lead to adverse outcomes, remains limited. This study employs a quantitative framework comprising an economic whole-farm optimization model and a GHG emissions calculation module to analyze how different decisions affect the economic and climatic performance of farms with various characteristics. The results show that decisions that can produce economic and climatic win-wins for one farm may lead to win-lose or lose-win outcomes for others, depending on farm-specific conditions and how farmers adjust other decisions based on changes in resource availability. Moreover, what represents a climatic win not only depends on farm circumstances but also on the chosen functional unit. These insights are relevant to policymakers, farm advisors, and farmers and can contribute to more targeted efforts to improve economic performance and reduce emissions in the cattle industry.

112 Modelling greenhouse gas emissions and mitigation strategies in Kenya's dairy system using life cycle assessment

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Kenya produces over 4.2 million tonnes of milk annually, but dairy greenhouse gas emissions (**GHGE**) account for 15% of national emissions. Milk demand is projected to rise by 85% by the year 2050, making effective mitigation strategies essential for sustainable dairy farming and reducing GHGE. This study aimed to model the effectiveness of various strategies in mitigating GHGE within Kenya's dairy cattle systems. Life cycle assessment methodology was used to calculate the carbon footprint (**CF**) of small-scale dairy systems and model mitigation intervention scenarios. The CF assessment followed a 100-year global warming potential framework with values of 27.2 for methane, 273 for nitrous oxide, and 1 for carbon dioxide. The system boundary was set to a "cradle-to-farm-gate" perspective. Allocation of emissions between milk and meat products was performed using both biophysical and protein allocation methods, with a functional unit of 1 kg of fat and protein-corrected milk (**FPCM**). Data was collected through surveys conducted with 96 smallholder farmers across Nandi and Uasin Gishu counties. Emission calculations followed the 2019 Refinement to 2006 IPCC guidelines incorporating local emission factors. Additionally, a validation workshop with farmers was held to review and confirm the proposed mitigation intervention scenarios. In each farm, 8 mitigation intervention scenarios were modeled: (S1) Improved feeding + 10% milk productivity increase; (S2) Improved feeding + 20% milk productivity increase; (S3) Male removal + improved feeding + 10% milk productivity increase; (S4) Male removal + improved feeding + 20% milk productivity increase; (S5) 50% manure in biodigester + improved feeding + 10% milk productivity increase; (S6) 90% manure in biodigester + improved feeding + 10% milk productivity increase; (S7) 50% manure in biodigester; (S8) 90% manure in biodigester. In the improved feeding intervention, natural pasture intake was reduced by 50% and replaced with *Urochloa* (*Brachiaria*) hybrid Mulato II for its superior nutritional characteristics. S1 to S6 were modeled with and without incorporating soil carbon sequestration at a rate of 18.28 t C ha⁻¹. A factor analysis for mixed data and hierarchical clustering on principal components were performed to classify farms into distinct clusters. Three farm clusters were identified: small scale (**SS**), medium scale (**MS**), and large scale (**LS**). The main sources of GHGE were enteric fermentation and manure management. Baseline CF varied among clusters, ranging from 1.42 to 22.09 kg CO₂e kgFPCM⁻¹ and from 3.97 to 39.48 kg CO₂e kgFPCM⁻¹ of IDF and protein allocation rules, respectively. SS achieved higher milk productivity and the lowest CF values (3.02 kg CO₂e kgFPCM⁻¹) compared to MS (4.45 kg CO₂e kgFPCM⁻¹) and LS (4.95 kg CO₂e kgFPCM⁻¹). Mitigation interventions could reduce 14 to 33% CF among clusters. Scenarios that included soil carbon sequestration from improved forage could reduce an additional 10 to 18% of CF. This study shows the potential to reduce GHGE in Kenya's dairy cattle systems through improved feeding, increased productivity, herd management, manure handling, and soil carbon sequestration. Variations across farms highlight the need for tailored strategies, with farmer validation ensuring practical, sustainable solutions aligned with climate goals.

164 The costs and benefits of different mitigation interventions in rural production systems in Africa

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Livestock are deemed responsible for about half of Africa's methane emissions (0.6 Gt CO₂eq), and account for a bit more than 1% of global annual anthropogenic GHG emissions. Despite this low contribution, the potential for reducing emissions per animal and related to milk, meat, and eggs is enormous, due to the low productivity compared with other systems. There are considerable investments in mitigation interventions in rural production systems in Africa from philanthropy, international agencies, and inter-governmental funds targeting GHG reduction.

Technical interventions include improvements in feed quality and availability, improvements in animal health and breeding, and promotion of relatively new technologies such as the use of methane inhibitors and vaccines that are aimed at reducing emissions from enteric fermentations through different pathways.

The uptake of these interventions and the potential GHG reduction will depend on economic considerations, management practices, and local conditions.

Using literature data and carbon costs, we have developed a series of scenarios to explore the cost and benefits of different mitigation strategies. The results of this assessment can guide the prioritization of investments in GHG reduction focused targeting the domestic ruminant livestock population in Africa. This analysis will therefore put the potential GHG emission abatement into perspective with mitigation efforts and potentials in other sectors and regions.

222 HOLOS-IE digital platform for carbon footprint assessment: a dairy farm case study

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The agricultural systems significantly contribute to greenhouse gas (GHG) emissions, primarily through enteric and manure methane (CH₄) from livestock and fertilizer-induced nitrous oxide (N₂O) emissions from grassland soils. To mitigate climate change, strategies such as dietary adjustments and feed additives for livestock and fertilisation with a circular approach to crops are important. Agroforestry has added advantages to offset GHGs through carbon sequestration while improving soil health and ecosystem services. To achieve ambitious climate goals, achieving carbon-neutral farms by 2050, agricultural production requires a sustainable transformation. System-based modelling has a pivotal role in providing a comprehensive understanding of agriculture, facilitating informed decision-making while effectively balancing data requirements. Accordingly, HOLOS-IE, leading to HOLOSEU, development aims to simplify complex modelling for farmers and other stakeholders, enabling them to reduce their agri-environmental footprint and achieve sustainable production.

The HOLOS-IE v3.0 (www.ucd.ie/holos-ie) leverages large datasets, evidence-based algorithms, gap-filling using GIS and Machine Learning, and coding using C#.NET. The ongoing development focuses on refining model components (crops, grasses, livestock, agroforestry and farm infrastructure), and their sub-components. These components are driven by key soil, climate and relevant variables, which are automated or user-defined inputs. As a preliminary case study, we applied the HOLOS-IE on a 30-hectare Irish dairy farm to examine alternative land use scenarios within an agroforestry system (silvopastoral configurations featuring Oak and Sycamore as hedgerows), by sparing 5% of the total land area without reducing livestock density. We evaluated the model's ability to predict sectoral GHG emissions, carbon removal, and total/net carbon balance. We analysed the results and quantified the offsetting potential through soil and biomass carbon sequestration. This analysis provided insights into the total and net carbon balance, informing future land-use planning for climate change mitigation.

The model successfully simulated sectoral outputs on GHGs, soil organic (SOC) and biomass carbon, and farm energy. In the dairy farm, the key contributor was enteric CH₄ (76% i.e. 5148 tCO₂eq ha⁻¹), direct N₂O (13%) and Manure CH₄ (9%), and the contribution of indirect N₂O was 2%, respectively. The change in SOC density in grassland was 0.16 t C ha⁻¹y⁻¹ on average over 23 years. Following the introduction of silvopasture, the grassland contribution to sectoral GHGs remained similar, but a sharp increase in SOC density was observed in the tree zone, significantly higher in hedgerows than in the silvopasture. Without compromising livestock density, sparing 5% of land area for the trees (silvopasture + hedgerows) accounted for offsetting 19% of the total dairy farm carbon footprint, enabling to adoption of further choices to make a farm carbon neutral. This paper introduces HOLOS-IE as a foundational step towards the development of HOLOSEU. As the model is still under development, a relatively comprehensive scenario demonstrating how to achieve carbon neutrality including soil health indices, production metrics, and cost-benefit analyses will be presented at the conference. Feedback from stakeholders will be gathered to guide further improvements, followed by validation and calibration targeting for HOLOSEU.

190 The GLEAM Global Livestock Production Systems (GLEAM-GLiPS)

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Livestock production systems (**LPS**) exhibit significant diversity worldwide, shaped by variations in resource availability, management practices, and environmental conditions. This diversity is mirrored in the unique challenges each system faces, necessitating targeted support through well-designed policies. To guide policy decisions effectively, it is essential to use robust frameworks to classify LPS. These frameworks enable the identification and prioritization of system-specific challenges and facilitate the development of tailored measures to address them. By incorporating these measures into policies, such frameworks can drive improvements in productivity, enhance income generation, and reduce environmental impacts, supporting the sustainable transformation of livestock systems. Existing approaches, often rely on external factors such as agro-ecological zones or resource use, inconsistent combinations of criteria (income sources and feed), or are partly based on outdated data. The objective of this work is to develop and test a flexible, hierarchical, and widely applicable LPS classification framework to support livestock and environmental assessments using the Global Livestock Environmental Assessment Model (**GLEAM**). The developed framework provides a starting point for systematically grouping LPS based on common management practices, such as feed use, and production goals. We demonstrate initial results of the global classification for different species using available data for feed basket composition and livestock management. Specifically, for the first node of LPS classification, we tested different thresholds for the fraction of feed derived from grazing. These thresholds and the outcome of our classification were compared and validated against classification systems employed in different countries. In addition, we demonstrate the subsequent nodes of the hierarchical system which incorporates herd orientation and other criteria through case studies covering different species across different regions and systems. Future work will focus on extending the framework with other classification criteria, reinforcing GLEAM's capacity to support sustainable livestock production, inform policy development, and contribute to equitable and environmentally sustainable food systems.

308 Assessing the input variables for CO₂-ekv per kg FPCM in Norwegian dairy farming using PLS

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The collaborative initiative, “Climate Smart Agriculture”, off the Norwegian agricultural sector has led to the development of an innovative greenhouse gas (**GHG**). This tool is designed to streamline data collection by integrating automatically collected inputs from multiple high-quality data sources, requiring minimal effort from farmers, who are only required to give their consent. The HolosNorMilk, based on the Canadian Holos, is tailored for Norwegian dual-purpose milk and meat production and provides the basis for the GHG calculator. GHG emissions are calculated according to the IPCC methodology, adjusted for Norwegian conditions where applicable (IPCC, 2019). The model calculates direct methane (**CH₄**) emissions from enteric fermentation and manure management, direct and indirect nitrous oxide (**N₂O**) emissions from manure management and soil, and carbon dioxide (**CO₂**) emissions from the production and use of farm inputs. The objective of this study was to analyse data from the GHG-calculator (N = 800 farms, year 2023) to assess the variation in the emission intensity of kg fat- and protein corrected milk (**FPCM**) in Norway and identify the key input variables contributing to the variation in the emission intensity using Partial Least Square (**PLS**) method.

Preliminary results revealed significant variation in the emission intensities among farms, ranging from 0.7 to 1.4 CO₂-ekv per kg FPCM. The most influential input variables contributing to this variation in emission intensity per kg FPCM are the net energy for lactation, the average live weight of the cows, and the proportion of pregnant cows in the herd. The amount of dry matter (**DM**) from concentrates and forage did not significantly contribute to the variation in emission intensity. A possible explanation is that measuring feed intake solely in kilograms of DM may not capture differences neither in the nutritional value nor the digestibility of the feed, both of which are critical factors influencing methane production and overall emissions. Currently, DM intake is estimated based on yield, live weight, daily weight gain and lactation. Consequently, the method used to calculate emissions may not fully capture the variations in emission intensity associated with different feed types. Further, key variables such as net energy for lactation, live weight, and pregnancy rate appeared to have a stronger influence on the emission intensity due to the correlation with feed intake, potentially overshadowing the direct impact of feed intake in the analysis. While the CH₄-emissions remains the largest contributor to the GHG-emissions from dairy cows, at the farm level other sources such as N₂O and CO₂, are significant. Therefore, the observed variation in emission intensities at farm level cannot be solely attributed to CH₄ emissions.

The study reveals that the cows' energy needs, including lactation energy, live weight, and pregnancy rate, are the primary drivers of variation in emission intensities on Norwegian dairy farms. However, improved data collection on feed and nutritional profiles could yield different results, as feed is known to be a significant factor influencing emission intensities.

141 Advancing the denitrification and decomposition model for biosolids and manure applied to cropland

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The objective of this study was to improve and validate the Denitrification and Decomposition (**DNDC**) model for simulating CO₂ emissions from land application of biosolids and manure. A separate manure C pool was added to the DNDC framework to disaggregate manure decomposition from the soil organic matter pools. Decomposition rates of biosolids were estimated using measurements of organic material. The effect of soil temperature on soil organic matter decomposition was also improved using an arctangent function. Data collected from two climatically distinct sites in Montreal (Quebec) and Truro (Nova Scotia) in 2017–2019 with continuous corn were used to test the model in simulating crop yield, soil temperature and moisture, and CO₂ fluxes from soil amended with biosolids (mesophilic anaerobically digested, composted, or alkaline-stabilized), urea, and an unfertilized control. A third site in Harrow (Ontario) (2012-2015) was used to verify the model for solid cattle manure and inorganic fertilizer applied to a field in corn-soybean rotation. Both the default and improved models were calibrated using data from the Ontario plots with inorganic fertilizer, and the surface spread treatments at Montreal and Truro, while the solid cattle manure, soil-incorporated and control treatments were used for validation. Crop yields were well simulated by the improved model as per the root-mean-squared error (**rRMSE** 4.1 - 30.1%) for all sites. The improved DNDC model (Wilmott d coefficient $0.72 \leq d \leq 0.96$) outperformed the default version ($0.61 \leq d \leq 0.9$) in simulating CO₂ fluxes across all sites. Similarly, the model effectively simulated both soil temperature ($d \geq 0.88$) and moisture ($0.53 \leq d \leq 0.91$) for all sites. The addition of an independent biosolids/manure C pool in DNDC resulted in more accurate simulation of decomposition rates for alkalized and composted biosolids to and of CO₂ emissions. The modified temperature function alleviated the over-prediction of CO₂ emissions shortly after biosolid application and greatly improved the timing of emissions during the growing season. The enhanced model will help simulate best management practices for integrated crop-livestock-manure systems, reducing reliance on nitrogen fertilizer.

244 Mechanistic modelling of 3-nitrooxypropanol in dairy cattle: mode of action on methanogenesis and fermentation

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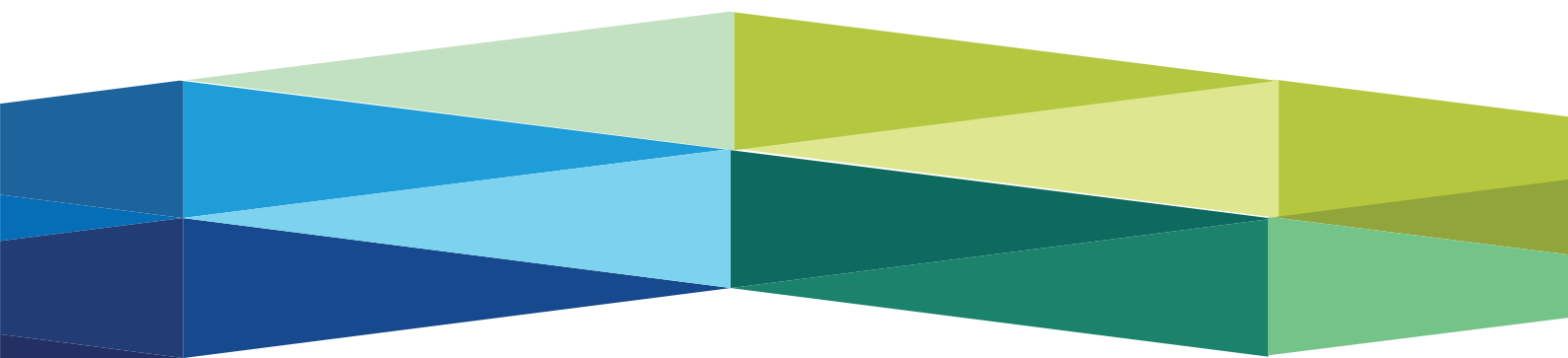
Global agreements aim to reduce greenhouse gas (**GHG**) emissions in the coming years. The feed additive 3-nitrooxypropanol (**3-NOP**) has emerged as a promising strategy, capable of reducing enteric methane emissions in dairy cattle by up to 60%. The effectiveness of 3-NOP in reducing methane production is variable and multifactorial, influenced by dosage level, diet characteristics, and composition of methanogen populations, amongst others. Accurate prediction of methane-mitigation effects of 3-NOP under varying conditions remains a challenge. Mechanistic modelling of the mode of action of 3-NOP in the rumen can enhance understanding and prediction of variability in 3-NOP effectiveness and its impact on rumen metabolism. Improved predictive accuracy facilitates optimizing the use of 3-NOP under specific feeding and production conditions. Compared with empirical models, the mechanistic modelling approach allows further and more in-depth examination of causal processes underlying the mode of action of 3-NOP. The objective of this study was to develop a mechanistic model that represents (i) the microbial fermentation of nutrients and the subsequent formation of end products including volatile fatty acids, hydrogen, and ammonia, (ii) the metabolism of methanogens that utilise hydrogen to produce methane, and (iii) the mode of action of 3-NOP in the rumen. In the model, the concentration of 3-NOP in the rumen depends on its rate of intake, rate of absorption and passage, and rate of degradation by microbes. The model describes the activity status of methyl coenzyme-M reductase (**MCR**), the key enzyme involved in methanogenesis that is inhibited by 3-NOP, subsequently affecting the rate of methane production. MCR activity is represented in two possible states, i.e., a physiologically active and inactive state, whose dynamics depend on 3-NOP concentration. Methanogen dynamics are represented mainly in response to fluctuating hydrogen levels. The model was developed using data from 37 experimental treatments from 15 published studies with dairy cows. Preliminary results show that the model is able to predict the observed range of methane mitigation by 3-NOP, ranging from 10% to 60% reduction, related to dose level and characteristics of the basal diet such as the ratio between forage and concentrate. The model simulates the consequences of 3-NOP for rumen metabolism, with increased hydrogen abundance upon greater 3-NOP levels. This shift causes a decreased proportion of acetate and increased proportions of propionate and butyrate, in turn affecting hydrogen production in the rumen. In summary, the mechanistic model provides a comprehensive representation of the activity of 3-NOP in the rumen, predicting its methane mitigation effects as well as its impact on rumen fermentation. It offers a valuable tool to optimize the mitigating effect of 3-NOP under varying conditions, based on the mechanistic understanding of the underlying processes.



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International Greenhouse
Gas & Animal Agriculture
Conference

Theme 8: National greenhouse gas inventory and policy frameworks with carbon finance mechanisms



319 How do climate policies impact animal welfare? An analysis of Africa's NDCs

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The livestock sector accounts for 12-20% of total anthropogenic GHG emissions and reducing their emissions is crucial for achieving Paris Agreement goals. In the previous round of updates in 2022, 88 countries included livestock measures in their nationally determined contributions. Due to the complexity of the production chain and emission sources within the sector, mitigation and adaptation measures vary strongly depending on national contexts and strategies. This study aims to analyse how climate measures in the livestock sector may affect animal husbandry practices and possibly impact animal welfare. Using a combination of quantitative and qualitative methods, the scope of this study focused on the African NDCs, sourced from the UNFCCC database, as the continent has a large diversity of livestock systems and a projected increase in demand for meat and dairy products. First, a quantitative analysis of the updated NDCs from 54 African countries was conducted, followed by a qualitative analysis to verify the impact of the measures listed in the documents. 12 measures were identified as potential impact factors based on literature reviews and were categorized as favorable, neutral and unfavorable to animal welfare. Seven countries that did not include livestock mitigation or adaptation actions within their NDCs were excluded from the qualitative analyses. The Spearman's correlation method ($p < 0.05$) was used to verify the correlation between potential impacts. Of the 54 NDCs analysed, 50% of countries included livestock in mitigation actions and 83% in adaptation actions. Seven climate mitigation and adaptation measures were identified as favorable impact factors to animal welfare, including grazing management practices (43%), enhancing farmer livelihood (37%), agroecological practices (54%), and linking climate and biodiversity measures (70%). Four measures were identified as unfavorable to animal welfare, including the intensification of livestock (33%), increased production of monogastric species (13%) and breeding programs for high productivity with an increased practice of artificial insemination (9%). Manure management was identified as a neutral impact factor, as practices and their effect on animals and housing conditions vary. The qualitative analysis showed a positive correlation between the impact on animal welfare and the inclusion of measures to enhance farmer livelihood ($r = 0.510, p < 0.001$), grazing management practices ($r = 0.450, p < 0.001$), and the intensification of livestock ($r = 0.634, p < 0.001$). This suggests that NDCs that include these measures tend to have the highest impact on animal welfare. Results show an overall higher inclusion of climate mitigation and adaptation measures that are favorable to animal welfare. However, significant risks for animal welfare, such as shifting to increased monogastric production and the intensification of animal farming are still often included in the NDCs. Additional potential trade-offs include increased risk of pathogen spread, AMR, and nutrient pollution. As a following step, the exercise of identifying and categorizing impact factors will be performed by a larger pool of interdisciplinary livestock and climate experts, specifically from the African continent. This study will preface a larger discussion on policy frameworks at the regional and national level, and how to minimize trade-offs and adapt to local contexts when implementing climate strategies within the livestock sector.

140 Institutional arrangements in rangeland carbon projects: a case study from Kenya

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The growing focus on mitigating greenhouse gas emissions through improved agricultural practices has increased interest in rangeland carbon sequestration projects. These projects contribute to climate change mitigation, offer community benefits, and promote biodiversity conservation. However, the success of rangeland carbon projects largely depends on the complex interplay of stakeholders, their roles, and influence within the project ecosystem. Considering the complexities and uniqueness of rangelands in Africa, knowledge about how these actors are organised to achieve favourable outcomes is crucial for identifying strategic approaches for successfully implementing rangeland carbon projects. Previous studies have examined community-based organisations in rangeland management in Africa, including the co-management of socioeconomic and ecological factors that are challenging development in Kenya's northern rangelands. However, where project implementation is guided by global standards and incentives, there is limited knowledge of the institutional conditions that enable the achievement of project outcomes. Drawing on the case of the Northern Kenya Grassland Carbon Project, this research examines the role of key stakeholders involved in the project implementation. Through Key Informant Interviews and Focus Group Discussions conducted with project leaders and participating project conservancies, the study applies the Net-Map—a participatory mapping method—to identify the project actors, understand their interlinkages and their influence on project outcomes. The expected results will be presented as a visual network diagram showing key actor categories and their perceived influence on project outcomes. The diagram will focus on actor linkages along four focus areas, which are contractual arrangements, authority, information, and fund (project and non-project funds) flows. The study will also include narrative insights into the governance challenges or opportunities along different actor linkages. We anticipate uneven control of resources within the project and a limited influence of local communities on project outcomes. This research contributes to the broader discourse on integrated climate and biodiversity solutions, emphasising the need for participatory stakeholder engagement in the governance of carbon offset projects. It provides insights into how inclusive stakeholder networks can enhance climate mitigation efforts, long-term sustainability and scalability in rangelands. Understanding these dynamics is crucial for designing more effective governance structures that balance carbon sequestration goals with socio-economic benefits and biodiversity conservation in future.

87 Assessing enteric methane emission standards for carbon crediting: Implications for forage-based systems.

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Ruminants are essential to global food systems, especially in smallholder farming, but they significantly contribute to methane emissions. As demand for livestock products grows, reducing methane emissions is crucial for sustainable production. This requires robust and cost-effective monitoring, reporting and verification (**MRV**) systems for transparency, climate finance, and scaling low-emission practices. The Low-Methane Forages (**LMF**) project, led by three CGIAR Centers (Alliance Bioversity-CIAT, ICARDA and ILRI), addresses these challenges by promoting anti-methanogenic forages, breeding methane-reducing traits, and deploying these solutions in livestock systems across the Global South. We screened twenty methodologies to quantify and certify enteric methane emissions from livestock systems, mainly supplemented with feed or additives. We then focused on four methodologies: Verra's Verified Carbon Standard (**VCS**) VM0041 and VM0042, Clean Development Mechanism (**CDM**) AMS-III.BK, and Gold Standard (**GS**). We evaluated their robustness, cost-effectiveness, scalability, permanence, additionality, co-benefits and applicability to Global South. Each methodology targets specific livestock production contexts. VCS-VM0041 focuses on methane reductions through feed supplementation in beef cattle, while AMS-III.BK addresses feed interventions in smallholder dairy systems. VCS-VM0042 incorporates carbon sequestration through improved agricultural land management practices and GS emphasizes enteric methane reduction via feed ingredients. These methodologies aim to improve feeding systems and reduce methane emissions per unit of product, like milk or meat. Their system boundaries vary, covering ingredient production, transport, and farm activities. VCS-VM0041 evaluates feed supplement impacts from production to on-farm application, while AMS-III.BK considers the full lifecycle of dairy animals in smallholder systems. The four methodologies offer varying approaches to quantifying enteric methane emissions, balancing accuracy, cost, and scalability. Direct measurement methods, like respiration chambers (VM0041) are accurate but resource-intensive, limiting their feasibility in low- and middle-income countries. Simpler methods, like IPCC Tier 1 factors or literature estimates (VM0042), are cost-effective but less precise. Mid-level approaches, like IPCC Tier 2 and meta-analysis, balance cost and specificity using detailed data and existing research. CDM's regression-based method links productivity with emission factors, offering tailored estimates but requiring extensive data. Therefore, the choice of methodology depends on project goals, balancing accuracy, cost and scalability. In resource-limited regions, like Global South, an affordable and scalable approach that ensures accurate accounting and credit integrity is recommended. IPCC Tier 2 equations (used in VM0042 and GS) offer accurate estimates without the high costs of direct measurements. This approach uses farm-level data to estimate methane reductions from improved pastures, making it feasible for large-scale and low-input systems. It also boosts the credibility of pasture quality improvements in carbon finance projects and national GHG inventories, supporting climate mitigation in pasture-dominated regions. This analysis highlights the potential of hybrid MRV methodologies, combining IPCC Tier 2 emission factors and peer-reviewed data, as scalable, cost-effective solutions for estimating methane reductions in improved pasture systems in the Global South. All methodologies met criteria for additionality and permanence, key Core Carbon Principles, making them attractive to potential buyers. These approaches provide the accuracy needed for carbon markets while remaining feasible in resource-limited contexts, enabling access climate finance and promoting sustainable practices.

130 Bibliographic mapping of research on greenhouse gases from livestock in Africa

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Greenhouse gas emissions from livestock pose a significant challenge to sustainable agriculture. This study analysed bibliographic data on this issue to identify key contributors and collaborations, with policy and scientific advancement implications. Bibliographic data were collected using Scopus, focusing on papers referencing greenhouse gases from African livestock. The collected documents were exported to Vosviewer and SciVal for analysis. The study covered publications from 1989 to 2024, assessing institutional contributions, funding sources, and collaboration patterns. Keywords, citation impacts, and publication sources were analysed to identify trends and influential works in the field. A total of 861 documents were identified, with the number of publications peaking at 118 in 2023. Major research institutions included ILRI, Wageningen University, and CSIRO, with the leading country affiliations being the USA, Kenya, and South Africa. The European Commission, the Consortium of International Agricultural Research Centres, USAID, and the International Fund for Agricultural Development primarily funded this research. The Field Weighted Citation Impact of the papers was 2.17, with 76% of the publications categorised under Natural Sciences. Collaboration was a significant factor, with 63% of papers from international partnerships and 3% from corporate partnerships. When policy bodies utilised these documents (33.6%), their citation impact increased to 4.0. Organisations engaging in this research included FAO, CGIAR, the UN Environment Programme, the European Union, and the World Bank. Livestock species covered were mainly dairy and beef cattle as the primary focus, followed by sheep. The dataset comprised 3,830 authors, with 56 publishing more than five papers. The authors in the dataset were included in six clusters out of nine across 150 countries. There were 2,359 keywords such as greenhouse gas, climate change, methane, mitigation, carbon sequestration, and environmental impacts. Of these, 93 appear more than five times. Of the 454 sources, the most prominent included the South African Journal of Animal Science, Science of the Total Environment, and the Journal of Cleaner Production. Prominent authors included Mario Herrero, Chris Zielinski, and Lutz Merbold. The papers referenced 53,833 works, with notable citations to Alexandratos & Bruinsma (2012), Gerber et al. (2016), and the COP26 special report. References were distributed across 19,056 sources, with 335 contributing more than 20 references, such as Nature, Science, and Animals. Bibliometric analysis reveals key advancements and collaborations in greenhouse gas research related to African livestock. International collaboration highlights the global significance of this issue, while strong policy impact shows the practical importance of the research. Funding from international organizations underscores the need for sustainable farming in Africa. The focus is primarily on dairy and beef cattle, indicating potential for further research on other livestock. This study identifies key contributors, funding sources, and collaboration networks, emphasizing the need for policy integration to enhance research outcomes. These insights can guide future research and inform policies to tackle environmental challenges caused by livestock in Africa.

366 Direct measurement of methane leakages from household biogas digesters in Sub-Saharan Africa

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Low- and middle-income African countries heavily rely on default IPCC values to estimate greenhouse gas (**GHG**) emissions reductions from manure-digesting biodigesters on smallholder farms. With over 100,000 installed digesters and ambitious expansion targets across Sub-Saharan African (**SSA**) countries, where many digesters generate carbon credits for offsetting emissions, robust quantification of GHG reductions is critical to ensure the credibility of these credits. However, there is a lack of localized or region-specific data on methane (**CH₄**) emissions from physical leakages of digesters and CH₄ losses from digestate storage systems.

This study addresses this critical knowledge gap by directly measuring CH₄ leakages from household digesters for the first time in SSA, aiming to provide accurate GHG emissions data and assess the carbon offset potential of biogas digesters in SSA livestock systems. Four representative smallholder biogas digesters (two prefabricated and two fixed-dome digesters) installed at the Mazingira Centre research facility, hosted by the International Livestock Research Institute in Nairobi, Kenya, were monitored for this study. These two types of digesters represent typical systems used for livestock manure-fed household digesters on smallholder SSA farms. All digesters were fed the same amount of a mixture of cattle manure and water under feeding conditions employed by smallholder farmers.

Initial biogas leak sources were identified by sniffing various components of the digester system (main digester body, feed inlets, expansion chambers, biogas lines, biogas burners, and digestate storage pits) with a Picarro cavity ring-down spectroscopy (**CRDS**) gas analyzer. For durable and well-manufactured digesters, the main leak sources were found to be feed inlets, expansion chambers, digestate storage pits, biogas lines, and biogas stoves. To estimate fugitive and vented CH₄ emissions from these sources, the closed chamber method coupled with CRDS was employed. In cases of unintended physical damage to digesters, methane venting was monitored in real-time using digital gas pressure and flow meters. Additionally, CH₄ and biogas (CO₂ and CH₄) production from the digesters were measured daily using diaphragm gas meters and portable biogas analyzers. Routine physicochemical and compositional analyses of manure and digestate samples were conducted to assess biodigester performance. Data analysis is ongoing, with results expected by March 2025.

The findings of this study are anticipated to provide localized and robust data on methane emissions from various digester components and activities for use in carbon crediting programs. These results will enhance more accurate estimates of the emissions reduction potential of biodigesters, inform interventions to mitigate unintended methane leakage, and improve national GHG inventories and credible carbon credit generation for livestock manure management systems.

281 Novel online tool for locating research facilities and analyzing enteric methane data from Sub-Saharan Africa

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Livestock in sub-Saharan Africa is of great importance in terms of food security, economy and socio-culture. Despite this, research data on enteric methane emissions by ruminants, a hot topic in livestock production, is incomplete and does not consider the diversity of existing production systems. Research data is usually not linked between different production systems or main producing regions. Furthermore, there is no centralised information system, which acts as a barrier to the development of strategies to improve livestock productivity while addressing climate change concerns. To address this issue, we created a web platform as part of the "Transforming Food Systems and Agriculture through Research in Partnership with Africa" (**TSARA**) initiative. This platform uniformizes and centralises data related to enteric methane emission, diets and animal performances, namely the variables measured during the study. In addition, the experimental resources available at the institution conducting the experiment are detailed. The web platform allows users to submit data ensuring consistency by checking data and it provides ways to compare it with the existing database. The web platform consists of two parts. One is called **MeCLAN**, which stands for the initiative "Method to evaluate low-Carbon Livestock in sub-Saharan Africa-research Network". MeCLAN application is a complete information resource from sub-Saharan African institutions. It is organised into seven sections: general information, location, additional information, ethics, land and livestock management, research topics and equipments. This data can be used to identify potential project partners. The second part of the web platform is a link to a web application called "MeTheix". MeTheix contains published experimental data including the experiments conducted by MeCLAN institutions. The collected data is organized into four main sections: variables related to the description of the animals; the diet composition fed to the animals; Nitrogen balance, digestibility and rumen fermentation parameters methane; and production (milk or meat) performance. Additional variables store the meta-data of the publication. MeTheix's data analysis allows to assess the impact of various dietary practices on enteric methane emissions. In addition to the data storage space provided via the website's database, MeTheix offers analysis tools and others such as custom graphs to visualize data and a keyword finder to identify relevant publications. By developing user-friendly intuitive interfaces, the website ensures that authorized users are able to visualize, upload, download, and analyze data. This website should facilitate collaborative research on agricultural greenhouse gas emissions reduction.

338 Advances in enteric methane emission estimation: greenhouse gas inventory improvement in Indonesia

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Greenhouse gas (**GHG**) inventories are a key tool to track progress toward global climate goals. The objective of this case study was to describe improvements in the Indonesian GHG inventory for enteric methane emissions from cattle production. The Indonesian GHG inventory began by applying the IPCC Tier 1 method (*Tier 1*) and has subsequently been improved using the IPCC Tier 2 method in two phases. In *Tier 2-phase 1*, the main improvement was to divide the livestock population into animal sub-categories based on age, sex or physiological status, and country-specific emission factors were developed for each sub-category. In *Tier 2-phase 2*, the cattle population was further divided into three livestock production systems (i.e. intensive, semi-intensive, and extensive systems), and emission factors were calculated using the IPCC Tier 2 predictive equations. In all phases, the activity data used in the inventory was gathered from the Indonesian Central Bureau of Statistics, the Indonesian Ministry of Agriculture, and literature reports. GHG emissions from enteric fermentation in 2022 estimated using the *Tier 2-phase 2* method (770,079 t CH₄ year⁻¹) show relatively small differences with calculations using the *Tier 1* method (827,319 t CH₄ year⁻¹, i.e. 6.9% higher than *Tier 2-phase 2*), but larger differences with the *Tier 2-phase 1* method (627,450 t CH₄ year⁻¹, i.e. 18.5% lower than *Tier 2-phase 2*). The main reason for the differences is that some major production systems in *Tier 2-phase 2* had higher emission factors (**EFs**) than *Tier 2-phase 1*. This reveals the effects of changing methodological approach between *Tier 2-phase 1* and *Tier 2-phase 2*: *Tier 2-phase 2* explicitly represented livestock sub-category populations in each production system, and the methane conversion factor (**Ym**) was calculated according to the type of feed used in each livestock production system. Monte Carlo simulation estimated enteric emission factor uncertainty in 2022 at (+16.0%, -14.1%), which was mainly driven by uncertainty of the Ym value, feed digestibility, and the coefficient for maintenance (**Cfi**) for adult females and yearlings in the intensive production system. These parameters should be priorities for collecting improved data in the future.

289 Livestock Data Catalogue: a living systematic resource to support livestock climate action

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There is ever increasing interest and intent for low- and middle- income countries to take action relating to livestock and climate change. NDCs are being updated with increased detail as to how targets will be reached. Whilst mechanisms are evolving for livestock development to benefit from climate finance, these efforts all require the input of robust data and evidence; which is typically elusive, scarce or disparate.

SEBI-Livestock are developing a living systematic method to map existing livestock data resources. As a living resource this is kept as relevant as possible through regular updates by repeating searches. This resource connects stakeholders with existing and available data resources; promoting the use of existing data, avoiding duplication of data collection and indicating real data gaps that deserve attention if climate ambitions are to be realised.

A living systematic approach means that the searches are rerun on a regular (at least annual) basis to check for changes or additions to the data resources available. Data resources are mapped using a standard search protocol which details data subjects known to be important in supporting climate action, such as livestock populations, production and productivity, animal health and nutrition.

Preliminary scoping searches focused on greenhouse gas emission data for livestock for Kenya, Tanzania, Ethiopia, Nigeria and India. Scoping searches identified data resources including bibliographic and grey literature databases, web-based search engines, and organisational databases; with a finding that most data concerning livestock GHG emissions were in grey literature reports and academic papers, rather than existing as accessible data files.

The Livestock Data Catalogue will provide a regular stock-take of the data resources available to decision makers. In response to the demand for action in the space of livestock and climate, this is where the first development of this resource has focused. However, longer term intentions are to make this living resource relevant to all areas of livestock development.

344 IPCC default enteric emission factor overestimates enteric methane emissions from growing-finishing pigs

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Intergovernmental Panel on Climate Change (**IPCC**) provides guidelines on methods for making estimates of greenhouse gas emissions from pigs. Following IPCC 2019 refined guidelines, the enteric methane (**CH₄**) emissions from pigs can be estimated by multiplying a default enteric CH₄ emission factor (**Y_m**) by the number of pigs. As of 2025, the Y_m factor for calculating enteric CH₄ emissions from pigs has not been updated since 1986. Consequently, the current methodology relies on data from a 40-year-old experiment with nine finishing pigs. Crutzen et al. (1986) proposed the Y_m factor for pigs to be 0.6% of the gross energy (**GE**) intake released as CH₄ across all pig subcategories. Since the Y_m is the same for all pig subcategories, it did not address variations in animal physiological stage and diet composition, which is especially important when considering diets for pigs in different subcategories that may have similar GE intake but varying nutrient profiles. The current study aimed to provide methodological updates on estimating enteric CH₄ emissions from growing-finishing pigs (30-150 kg) by updating the enteric Y_m factor for growing-finishing pigs. The dataset used to update the Y_m factor for growing-finishing pigs comprised two subsets. The first data subset contained 627 individual indirect calorimetry records on 197 growing-finishing pigs from seven published and thirteen unpublished studies conducted at Aarhus University, Denmark, between 1991 and 2024. The data subset was composed of individual enteric CH₄ production measurements on growing-finishing pigs along with the records on the body weight of the animals, the dietary concentration of GE, chemical compositions of the diet, and dry matter intake of the animals. The second data subset was found by a literature review of sixteen studies conducted between 1993 and 2018 that reported enteric CH₄ production measurements on growing-finishing pigs, where the fraction of GE intake released as CH₄ was either reported or could be calculated. The data subset obtained in the literature contained 95 treatment mean values (461 individual observations) on growing-finishing pigs. For each data subset, a weighted mean of Y_m was calculated using the number of individual observations per study as a weighting factor. Afterwards, a weighted mean of Y_m across both data subsets was calculated using the number of observations per data subset as a weighting factor. The weighted mean of the Y_m across studies performed at Aarhus University and those found in the literature were 0.40% and 0.56% of GE intake, respectively. The weighted mean of Y_m across all considered studies was 0.46% of GE intake, which is 23% lower than the currently applied Y_m factor for growing-finishing pigs. The current study's updated default enteric CH₄ emission factor for growing-finishing pigs more accurately reflects enteric CH₄ emission from growing-finishing pigs, thereby decreasing uncertainties associated with estimating enteric CH₄ emissions at national and global levels.

121 Variation and predictors of enteric methane emission factors for cattle in East and Southern Africa

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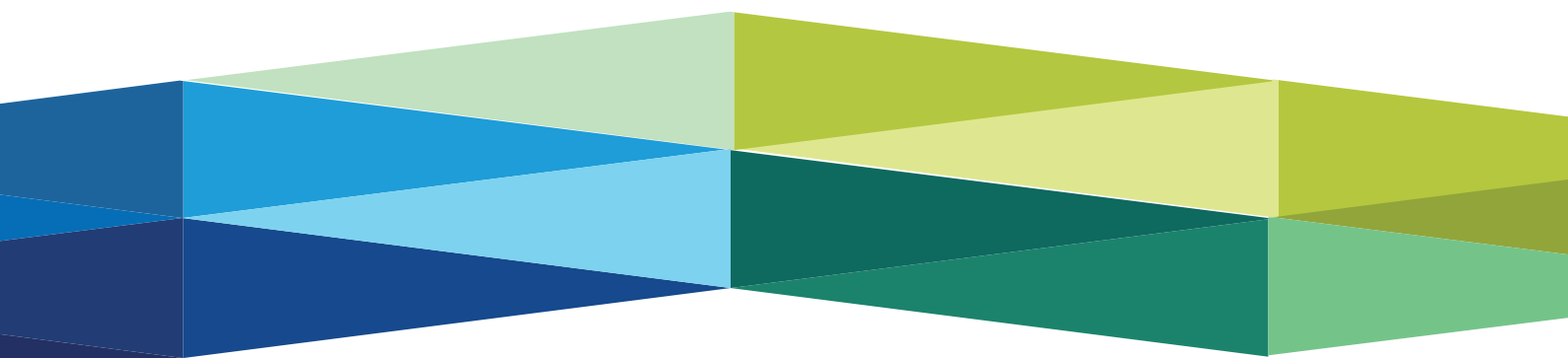
Countries in the East and Southern Africa (**ESA**) region have begun to improve national greenhouse gas (**GHG**) inventories of livestock emissions as a means to accelerate implementation of their climate change mitigation plans. Here, we review national Tier 2 GHG inventory reports for cattle emissions from dairy and other cattle production systems in ESA to evaluate variation in enteric methane emission factors (**eEF**) and their predictors, and highlight future priorities for improvement. We compiled 313 eEFs for dairy cows ($n = 26$) and other cattle sub categories ($n = 287$) estimated in 10 ESA countries' inventories using the IPCC 2006 and/or 2019 Refinement Tier 2 method and country-specific data. We also explored relationships between the activity data used as inputs in the IPCC equations and gross energy intake (**GEI**, MJ head⁻¹day⁻¹), a proxy of eEF, using simple and stepwise multiple linear regression to identify reliable predictors of eEFs. Enteric methane EFs for high (mean $\pm$ s.e.: 80.74 ± 2.19 kg CH₄ head⁻¹ year⁻¹) and low productivity (62.26 ± 0.97 kg CH₄ head⁻¹ year⁻¹) dairy cows were 6.5 and 4.5 % lower than the IPCC (2019) Tier 1a default EFs, respectively. Milk yield explained the greatest proportion of variation in GEI in dairy cows ($r^2 = 0.73$), and the regression function had a root mean square error of prediction (**RMSEP**) of 11.8 %. Notably, 8 countries included young and growing cattle in the inventory's dairy cattle category based on breed and management characteristics. Mean GEI for young and growing dairy cattle were significantly lower than for other cattle of the same age class in the same countries ($p < 0.05$), indicating the relevance of distinguishing dairy from other cattle breeds in ESA inventories. For low productivity other cattle, the eEF of 56.09 ± 1.0 kg CH₄ head⁻¹ year⁻¹, i.e., the population-weighted average eEF for young, growing and adult males and females, was 16 % higher than the IPCC (2019) Tier 1a default EF. Our eEF for high productivity systems (56.16 ± 1.0 kg CH₄ head⁻¹ year⁻¹) was 6 % lower than the IPCC (2019) eEF of 60 kg CH₄ head⁻¹ year⁻¹. Regression functions comprising liveweight (**LW**), average daily gain (**ADG**) and digestible energy explained 87 % of the variation in GEI in growing cattle (RMSEP = 8.1 %), and about 90 % of variation (RMSEP = 14.6 %) in young cattle. These findings highlight the need for ESA countries to obtain country-specific data for LW, ADG, milk yield and digestibility for estimating accurate EFs necessary in tracking GHG emissions in the livestock sector. Given that about 40 % of eEFs in the database were estimated using LW, ADG and/or diet composition data based on expert judgement applied to limited country-specific data, there is a widespread need for better data on these parameters across the ESA region.



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Theme 9: Measurement techniques for greenhouse gas emissions



363 Gap-filling algorithms and changes in carbon sequestration: eddy covariance measurements in a rangeland, Kenya

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Rising food and energy demand, combined with climate change, necessitates carbon sequestration, reforestation, and climate-smart agriculture. Despite its significant contribution to the global carbon cycle, Africa's ecosystem functioning changes remain little understood due to a lack of long-term in situ data. It is necessary to acquire continuous data and use the eddy covariance technique. This study aims to evaluate gap-filling algorithms for Eddy Covariance (**EC**) flux data and assess changes in carbon sequestration in Kenyan rangelands using machine learning methods. We also used eddy covariance to determine net ecosystem exchange (**NEE**), which includes gross primary production (**GPP**) and ecosystem respiration (**Reco**). This study compared gap-filling using the conventional approach, marginal distribution sampling (**MDS**), and artificial neural networks (**ANN**). The ANN machine learning algorithm performed well during training and validation, with $r = 0.86-0.98$, $R^2 = 0.74-0.93$, and $NSE = 0.74-0.93$ during training and $r = 0.91-0.99$ and $R^2 = 0.83-0.97$ during validation. Significant negative trends were found for NEE. NEE declined across the seasonal study period, with rainy season GPP showing larger negative trends than Reco. Increased atmospheric dryness could be one reason for the decline in carbon sink capacity. Rising temperatures in the rangeland, combined with higher evaporative demand, may result in GPP in this biome, limiting carbon dioxide sequestration capability.

379 A simplified headbox respiration system for measuring CO₂ and enteric methane emissions from cattle

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Increasingly there is a need to quantify enteric methane production by ruminant livestock in the many countries of Asia and Africa that rely heavily on ruminants for livelihood and food security. While the array of measurement tools is increasing, accurate direct methods to quantify total daily methane production at a low cost and without the need for strong technical skills or resources are not widely available.

A simplified headbox gas exchange system was built that relies on collection and analysis of a single pooled sample of exhaust air collected over 22-24h, as well as measurement of total air flow through the headbox. Typically, 4 headbox systems are built at each location. Key components of each headbox include [a] a side-channel blower capable of drawing $\geq 500\text{L}$ of air/minute [Ventex Model: 2RB 510-7AA21]; [b] a T junction with stopcock allowing headbox and external air streams into the blower to be managed differentially, thus enabling the flow through the headbox to be maintained at a target flow rate [c] a turbine flow-meter to quantify the airflow (Aichi tokei ATZTA TBZX). [d] a miniature peristaltic pump collecting a continuous sample of exhaust gas into a 5L TedlarTM bag at approximately 2 ml/min [Longerpump BQ50-1J-A]. [e] A calibrated gas analyser (either GC or infrared multi-gas analyser to quantify the gas concentrations). In all countries, small cylinders of CO₂ are available, so the simplest test for recovery of gas through the headbox was made by releasing gas from a CO₂ cylinder fitted with a single-stage regulator and a rotameter while placed on an electric balance with 1g resolution. The rate of change of weight of the cylinder was used to quantify CO₂ release over 3 minutes, while plateau CO₂ concentration in the headbox exhaust was monitored real time. Average air flow rate was recorded, then combined and CO₂ flux through the chamber expressed as a percentage of CO₂ release rate. Where pure CH₄ was available, a mass flow controller was used to release CH₄ rather than relying on the gravimetric determination of CO₂ release.

While air flow rates of 500L/min were standard, in high temperature and high humidity situations, a greater air flow was needed to avoid heat stress of cattle and condensation of water in the exhaust air ducting. Typically, air temperature in the headbox was up to 3°C higher than the ambient temperature.

These simple headbox system have allowed the first dynamic measures of methane emissions from cattle to be made in Cambodia, the Philippines and Lao PDR, providing a basis for improving the national livestock GHG inventory and livestock management. The methane yields of cattle fed nationally important diets in these countries and measured using these headboxes are in keeping with modeled estimates, typically being 19-22 g CH₄/kg Dry Matter intake, and provide evidence of the efficacy of nutritional mitigation of enteric methane.

332 WearME: a livestock-wearable methane emissions monitoring system

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Accurate measurements of methane produced by ruminants are fundamental for climate change strategies. Major uncertainties for on-field technologies reside in estimating the total volume of methane from an intermittent source. These technologies lack accurate calibration of the exhaled and captured volumes, especially for free-grazing animals. We present the design and validation of a wearable on-field methane sensing system and the development and verification of parametric and machine learning-based models to predict daily methane production. The sensing capability is provided by a laser-induced graphene electrochemical sensor (eLIG) capable of measuring methane concentrations between 1 and 3,000 ppm in near-real-time using differential pulse voltammetry. The sensor calibration curves are corrected for temperature and relative humidity, and demonstrate precise, accurate, and selective methane quantification, as corroborated via GC-MS referenced gas mixtures over the range of expected conditions. The developed methane sensor is integrated into a snout-wearable low-power IoT device, with embedded long-range wireless communication (via the LoRa protocol), 6-DOF inertial measurements (via an IMU), geolocation (via an 18 satellite GPS), and a hybrid piezo-electric and solar power management unit. Acceleration data from the IMU predict burping events, based on a supervised training machine-learning algorithm, to enable synchronous activation of sensing events during burping cycles. The device has been validated on a digital-twin ruminant respirator and on bovines. The digital twin allows in-lab testing with precise control over experimental conditions, including humidity, temperature, methane concentration, the volume of methane produced per respiration cycle, and head movement. To estimate daily methane production per individual, we developed a machine learning model to predict eructation events' onset, cessation, and concentration levels in a low-tropic environment with animals in various activities. The training set was constructed from 500 belching events in 300 specimens obtained in diverse environmental conditions and animal activities, using a high-precision laser methane detector and IMU data from our wearable device. This data set is then used to train a deep neural network to predict methane concentration levels from a 10-second signal of preceding data (motion and concentration). From these findings, we additionally build parametric models based on alpha, Gaussian, and lognormal functions to estimate total daily methane emissions per animal using the gas concentration data provided by our wearable IoT device. Our daily methane predictions are supported by understanding the fluid dynamics of respiration and eructation via Background-Oriented Schlieren (**BOS**) imaging and Large Eddy Simulations (**LES**) that capture the plume's momentum, transport, and dispersion characteristics. Our findings reveal that the belching flow is characterized by a two-stage jet mechanism: a starting jet, where the flow is initially released, and an interrupted jet, which follows the momentum source termination. Methane concentration emissions align closely with respiration chamber estimates. This work contributes to developing more effective strategies for mitigating greenhouse gas emissions by introducing a novel real-time livestock methane monitoring wearable device, fundamental knowledge of the fluid dynamics of ruminants' respiration, and physics-based methane emission prediction models for free-grazing livestock.

135 Variability in GreenFeed measurements from feedlot cattle

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The GreenFeed system is a popular tool for assessing enteric methane (**CH₄**) production from individual ruminants; however, there is a lack of standardisation regarding the operation and data analysis, as well as critical review of the data. Therefore, a study was conducted to assess CH₄ emissions using the GreenFeed system of feedlot steers consuming backgrounding and finishing diets. The study was structured as a 2 × 2 factorial, with two feed additive treatments (Control, Essential Oil) and two monensin treatments (no Monensin, Monensin). One-hundred Angus cross steers were evenly distributed across each treatment into four pens, and each dietary phase consisted of four, 28-d periods. Each pen was equipped with five Growsafe feed bunks to calculate dry matter intake (**DMI**), and two GreenFeed systems were held in two pens with 25 steers allocated per pen and GreenFeed unit. For CH₄ yield (g CH₄/kg DMI), the mean CV within the same steer across measurement periods was 14.0% and 15.2% for the backgrounding and finishing phases, respectively. The mean CV of CH₄ production for steer within period and time-of-day block was up to 83.4% and 102.9% for the backgrounding and finishing phases, respectively. The mean CV of number of visits per steer within period and time-of-day block was 34.7 and 42.7% for the backgrounding and finishing phases, respectively. There was an effect of period on CH₄ production, yield and CH₄ energy as a percentage of gross energy intake (Ym, Mcal CH₄ % gross energy intake). Number of GreenFeed visits was negatively ($P < 0.01$) correlated with all feeding behaviour metrics in the finishing phase. In conclusion, the high variability in CV of CH₄ production and yield suggests a need to improve the accuracy and precision of the GreenFeed system for estimating enteric CH₄ emissions from cattle. Additionally, the study highlights that the inclusion or exclusion of specific animals in the study can have a large impact on results due to large individual animal variability.

228 Inferring methane emissions from African livestock by fusing drone, tower, and satellite data

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Considerable uncertainty remains in the regional mapping of methane sources, especially within Africa's extensive agricultural regions. To address this issue, we have developed an observing system that estimates methane emission rates by assimilating drone and meteorological tower data into an atmospheric dispersion model. We show that our approach can be used to verify and quantify potential methane sources identified through radiance anomalies observed in satellite data. At the Kapiti Research Station in Kenya, we used our drone observing system to obtain emission estimates from various ruminant livestock, including Boran cattle (cows, heifers, steers), Holstein-Friesian and Boran crossbreeds, sheep (lactating ewes), goats (dry does, pregnant does, weaner kids), as well as camels (dromedaries), for which methane emission estimates are particularly sparse. In a parallel exploratory study, we analyzed hyperspectral data from the PRISMA satellite while three cattle herds were grazing at different locations. We detected anomalies in the methane absorption feature within the Shortwave Infrared region, corresponding to the herd positions. Although the limited dataset prevents a robust assessment, these preliminary findings enhance our confidence in the value of hyperspectral satellite data for detecting the location of potential methane sources. Using a drone equipped with a gas sensor, we collected methane concentration observations over a 20-minute period downwind of the livestock herds. By applying Bayesian inference, a probabilistic methodology, we updated our prior probability distributions over the methane emission rate and other uncertain parameters by incorporating newly acquired information. Fusing drone data and wind measurements from a nearby meteorological tower into an atmospheric dispersion model enabled us to constrain the probability distributions, resulting in more precise estimates of the herds' methane emission rates. Our Bayesian inference results demonstrate the hypothesized increase in methane emissions from ruminants after feeding. Our estimates align with Tier 2 emission values from the Intergovernmental Panel on Climate Change, indicating compatibility without implying mutual validation. Furthermore, when compared to a mass balance approach with drone observations - which assesses the methane emission rate by calculating the net inflow and outflow within a conceptual box enclosing the source - the Bayesian method proves more robust under non-stationary wind conditions. Furthermore, the Bayesian method performed better in quantifying emissions from weaker sources, estimating methane emission rates as low as 100 g h⁻¹, corresponding to sheep and goat herds of 100 to 200 animals. We obtained a posterior uncertainty of 50% in emission rate estimates for these herds, which is reduced to 12% for cattle herds of 150 to 200 animals, emitting 1,000-1,500 g h⁻¹. These findings demonstrate the potential and efficacy of the Bayesian inference method for source term estimation. Future applications of drone-based Bayesian inference could extend to estimating methane emissions from various sources with complex spatiotemporal emission patterns, such as wetlands, landfills, and wastewater disposal sites. In agricultural emission research, this method offers opportunities to investigate diurnal variations in emission rates and the effects of different feed intakes on emissions. The Bayesian observing system could thereby contribute to the validation and improvement of climate models and emission inventories.

274 Methane emissions from dairy cows: concordance between measurements from GreenFeed system and sulphur hexafluoride tracer

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Measuring enteric methane emissions from ruminants is essential to determine abatement potential of reduction strategies. The modified sulphur hexafluoride (**SF₆**) tracer technique has been shown to have a high concordance with open-circuit respiration chambers. Similar to chambers, the SF₆ technique has a relatively high labour requirement, although more animals can be measured in a given timeframe. The GreenFeed system (C-Lock Inc., Rapid City, USA) can estimate short-term emissions over an extended period, and with enough units does allow greater measurement capability with lower labour inputs. To our knowledge there has been no previous work undertaken comparing the two measurement techniques in Australian dairy systems where methane mitigants are offered twice daily at milking. The objective of this study was to determine the concordance between methane emissions, measured from dairy cows, using GreenFeed and SF₆.

Seventy multiparous, Holstein-Friesian cows were allocated to one of five methane mitigants (CON, MM1, MM2, MM3 and MM4) for a 63-day experimental period. The basal diet consisted of *ad libitum* vetch hay and ~6 kg DM/d grain. Mitigants were administered in the grain mix offered to each cow in individual feed bails during milking twice daily. GreenFeed methane measurements were obtained over 37 days (days 26 to 63) and compared with SF₆ measurements collected over five days (days 51 to 56). Data used included a minimum of 40 GreenFeed visits per cow and a corresponding SF₆ measurement. Data from SF₆ measures was deemed suitable for analysis if a particular cow had at least three days of good data. These criteria resulted in forty-six cow data points (CON: 10 cows, MM1: 8 cows, MM2: 11 cows, MM3: 8 cows and MM4: 9 cows) used in the analysis. Lin's Concordance Correlation Coefficients (Genstat 24) were used to determine the strength of concordance between measurements from the two methods.

Mean methane emissions measured using SF₆ versus GreenFeed were numerically consistently similar for CON (548 vs. 532 g/d), MM1 (508 vs. 503 g/d), MM2 (520 vs. 512 g/d), MM3 (447 vs. 429 g/d) and MM4 (581 vs. 543 g/d), but consistently lower with GreenFeed across all treatments, in comparison with SF₆. However, there was only a fair overall concordance between the two methods (0.521, $P < 0.05$).

Given that previous work has shown methane measurements from the modified SF₆ technique to be equivalent to measurements from open-circuit respiration chambers, the low concordance (< 0.80) between the two methods indicates that GreenFeed systems were not a suitable methane measurement technique in our experimental conditions.

386 Implementing the SF₆ technique for measuring methane from dairy cows under Fijian conditions

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Fiji currently uses Tier 1 methods from the IPCC to calculate the National Greenhouse Gas Inventory. However, a Tier 1 approach is not sensitive enough to capture methane mitigation strategies from livestock, beyond reducing animal numbers. Tier 2 methods can capture emission reduction strategies, but requires the development of country-specific Emission Factors. Determining an Emission Factor requires methane emissions from animals to be measured. The SF₆ technique was chosen in Fiji to measure methane emissions and to facilitate the development of a Fijian enteric methane Emission Factor for dairy cows.

SF₆ equipment was designed and constructed taking into consideration the animals proposed for the research, cost of equipment, and local resources. Where possible, equipment was sourced locally to facilitate repairs and design changes. Crimped capillary-tubes were chosen as the flow restrictors due to cost. The bias in sampling due to declining sampling rate with time was offset by using a tight range of initial flow rates (0.23 to 0.31 mL/min) and high initial canister vacuum (< 3.0 kPa absolute pressure). Permeation tubes (Agriculture Victoria Research, Ellinbank, Victoria, Australia) were calibrated by weighing twice per week for 4 weeks. Release rates ranged from 5.07 to 5.59 mg/day. Halters were a modified version of one commercially available, and the saddles for holding the sample canisters were designed and constructed in Fiji to suit the size and shape of the cows at the research station. Ten cows (6 Kiwi Cross, 4 Brown Swiss) were used to test the implementation of the SF₆ technique. Each cow was fitted with 1 breath-collection canister and 1 background canister. Samples were collected for 24 hours for 5 days.

Of the 100 samples scheduled, 1 cow sample and 2 background samples were lost due to equipment failure, and 1 sample was lost during analysis. There were more issues with equipment than lost samples, but by performing multiple checks per day, the equipment was able to be fixed and adjusted before sample collection was compromised. The saddle design was modified to reduce the risk of breakage. There was one cow that regurgitated her permeation tube, which prevented methane emissions being calculated for that cow. Methane emissions from the Kiwi Cross cows (5) averaged 259 g/d while the Brown Swiss cows (4) averaged 319 g/d.

The SF₆ technique was successfully implemented in Fiji to measure enteric methane from dairy cows. The technique shows promise as a way of determining the effect of mitigation options on methane, such as the effect of animal genetics, feed quality or feed additives. Measuring methane from additional animals should enable the calculation of country-specific emission factors which would enable Fiji to move from a Tier 1 accounting method to a Tier 2 method.

159 Quantifying enteric methane production from dairy cattle using GreenFeed in indoor and outdoor grass-based systems

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Quantifying enteric CH₄ production from dairy cattle is challenging due to, among other factors, diurnal emission patterns, which influence the accuracy of spot sampling techniques like the GreenFeed system (C-Lock Inc.), designed for short-term measurements in indoor and outdoor settings. The objective was to determine the minimum number of spot measurements per cow, relative to measurement duration, required to reliably quantify enteric CH₄ production from dairy cattle in indoor and outdoor systems. Data were collected from 2020 to 2023 at Dairy Campus (Leeuwarden, the Netherlands) from dairy cattle in various unrestricted (24 h/d except during milking) and restricted (8 h/d) grass-based systems, supplemented with 5.5 kg/d concentrate. In 2020 and 2021, grass-based systems included unrestricted grazing, zero-grazing, and grass silage diets, and restricted grazing on short or long pre-grazing sward height. In 2022 and 2023, grass-based systems included unrestricted grazing, restricted grazing, and restricted zero-grazing. All restricted grazing systems were supplemented with grass silage. Each year consisted of three periods: April-May (period 1), June-July (period 2) and August-September (period 3). Each period consisted of 2-week adaptation and 2-week measurement and was performed as a randomized complete block design with 15 or 16 cows per system. In total, 47,074 CH₄ spot measurements using GreenFeed units placed indoors and outdoors were obtained from 745 measurement units (*i.e.*, unique dairy cow × period × year combinations). Measurements were filtered based on a minimum duration of 2, 3, or 4 minutes. Simple random sampling without replacement was performed on the three filtered datasets including all cows with more than 30 spot measurements per 2-week measurement period using R software. For each spot measurement count, ranging from 1 to 30, we randomly selected spot measurements 100 times (replicates). The mean absolute percentage error (**MAPE**) was calculated for each replicate-spot measurement count combination. The MAPE was averaged per spot measurement count per grass-based system for further analysis. A linear mixed model analysis fitted using restricted maximum likelihood (**REML**) was conducted to test for differences in the minimum number of measurements needed across systems. A reliable estimate of CH₄ production was defined as a MAPE of less than 4% relative to the observed mean based on all spot measurements. Irrespective of measurement duration, a minimum of 16.2 measurements were needed for unrestricted grazing, 14.2 for restricted grazing, 9.7 for unrestricted zero-grazing, 12.4 for restricted zero-grazing, and 10.2 for unrestricted grass silage systems. Hence, irrespective of measurement duration, indoor systems required significantly less spot measurements ($P < 0.001$) than outdoor systems (11.0 versus 15.2, respectively). Averaging across all systems, a minimum of 14.4 measurements was required for measurement durations of at least 2 minutes, 12.9 measurements for durations of at least 3 minutes, and 11.7 measurements for durations of at least 4 minutes ($P < 0.001$). In conclusion, outdoor grass-based systems require more spot measurements with the GreenFeed system than indoor grass-based systems to obtain reliable enteric CH₄ production measurements from dairy cattle. Increasing the measurement duration decreases the minimum number of spot measurements needed, regardless of system.

138 Estimating enteric methane emission factors for cattle in Uganda using IPCC Tier 2 methodology

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Amidst the need for Many developing countries in Sub-Saharan Africa to increase production of animal source foods to meet nutritional demands for their populations, their National greenhouse-gas (**GHG**) inventories still use default IPCC Tier 1 GHG emission factors (**EFs**) to estimate enteric methane (**CH₄**) emissions from livestock. Because these EFs are based on data primarily from other regions especially global north, there is a high degree of uncertainty associated with CH₄ emission estimates from African livestock systems. Accurate Tier II GHG emission reporting from developing countries becomes particularly important following the Paris Climate agreement made at COP21, which encourages countries to mitigate GHG emissions from agricultural sources.

In light of this, the present study provides improved enteric CH₄ emission estimates for cattle in Isingiro, Kiruhura, and Kazo districts of Uganda representing the common livestock production systems (agro-pastoralism and ranching) in the Uganda's cattle corridor.

Using the data from measurements of live weight and live weight changes, milk production and locomotion collected from 1,896 cattle, we estimated gross energy intake. From this and assessments of digestibility from seasonally available feeds, we estimated feed intake and used this to calculate daily CH₄ production by season, and, subsequently, created EFs. Overall mean EFs were 92.2, 58.9, 45.2, 78.0, 50.3 and 23.6 kg CH₄/head/year for dairy cows (>3 years), pregnant heifers (1-3 years), non-pregnant heifers (1–3 years), adult males (>3 years), growing males (1-3 years) and calves (<1 year) respectively.

The Overall total CH₄ emissions were 45,000 kg/year using Tier 2 and 55,000 kg/year using Tier 1, representing a difference of -18.2%, emphasizing the need for localization based on region-specific data for more accurate emission estimates for national livestock GHG reporting and mitigation strategies for sustainable livestock production. The findings from this study will help improve local inventories, national livestock GHG reporting as well as aid making of informed mitigation strategies for sustainable livestock production.

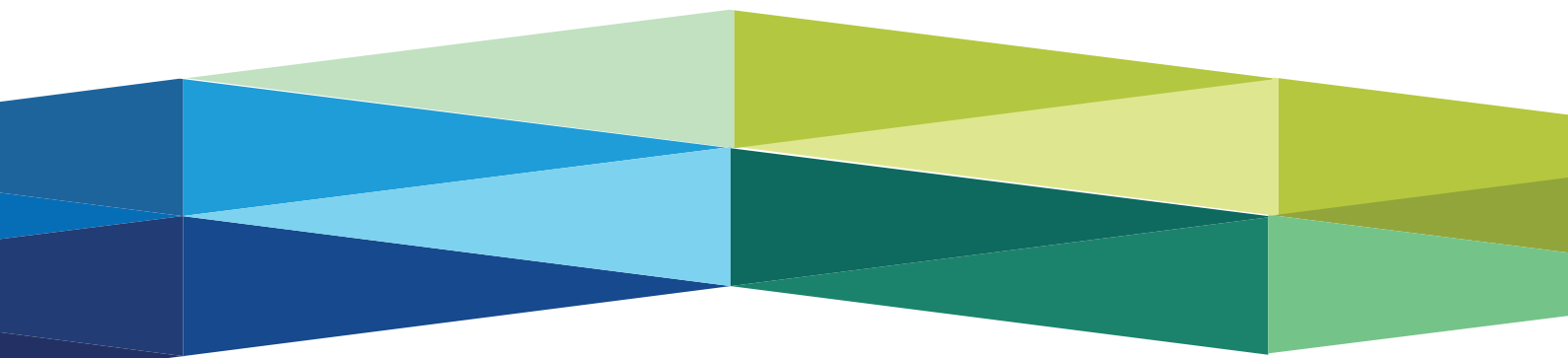


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Theme 1: Impact of animal feed and nutrition on greenhouse gas emissions



7 Determinants of farm-level Greenhouse gas reduction: Insights from the adopting climate-smart dairy strategies in Kenya

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In Kenya's dairy sector, climate change mitigation measures strive to intensify sustainable milk production. However, dairy producers often overlook emission reduction in their daily operations, creating a disconnect between the national environmental policies and local priorities. Therefore, developing measures that increase production while simultaneously reducing emissions is crucial. This study examines the effect of adopting climate-smart dairy strategies (improved breeding, improved feeds and feeding, animal health management and manure management practices) on reducing greenhouse gas emissions (**GHG**) and the factors that may influence the mitigation of GHG by using ordinary least squares. A survey of 385 farmers involved in dairy production was used. Based on the literature, we used proxies as indicators of greenhouse gas reduction. The greenhouse reduction was measured using a composite index. Accordingly, education, social capital, entrepreneurial orientation, awareness, and risk perception influence the adoption of Greenhouse gas reduction strategies. This study recommends that national and county governments promote and support the adoption of climate-smart dairy strategies that increase milk production while simultaneously reducing greenhouse gas emissions. This support could include providing technical assistance and educational programs to encourage complementary adoption by dairy farmers.

9 The nexus between livestock production, GHG emissions and feed production in Sub-Saharan Africa

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The livestock sector in Sub-Saharan Africa is critical for food security and economic development but contributes significantly to rising greenhouse gas (**GHG**) emissions, exacerbating environmental concerns. This study investigates the dynamic relationships between livestock production, GHG emissions, and feed production from 1970 to 2023, utilising a parsimonious vector autoregressive (**VAR**) model. Understanding these interactions is essential for developing policies that balance economic growth with sustainability. The VAR model, combined with stationarity tests and cointegration analysis, identifies both short- and long-term relationships among the variables. Impulse response functions (**IRFs**) and variance decomposition were employed to assess how shocks in one variable affect the others over time. The results reveal a bidirectional relationship between livestock production and GHG emissions, suggesting that increases in livestock output are tied to higher emissions. Feed production, while lagging, is a significant driver of livestock growth, with an increase in feed availability linked to a delayed but substantial reduction in GHG emissions due to improved feed efficiency. These findings emphasise the need for sustainable livestock practices. Policies should focus on enhancing feed production and efficiency, which can reduce emissions and bolster livestock systems. Climate-smart agricultural techniques such as rotational grazing and improved feed conversion rates are also crucial for balancing livestock growth and environmental protection.

27 Effect of red seaweed from the bay of Bengal on methane ranking in goats

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Methane (**CH₄**), a potent greenhouse gas (**GHG**), is one of the contributors to global warming and one of the main GHGs emitted by the ruminant livestock. The objective of the research was to determine whether red seaweed, *Asparagopsis taxiformis*, from the Bay of Bengal reduced CH₄ emission in goats. Using a cross-over study design, four goats were randomly allocated to two groups and one group was fed concentrate feed with red seaweed (1%) while the other group (control) was fed concentrate feed only. The treatments were swapped between goats before a second measurement period after one week for washing out. The goats were fed 500 g of concentrate per day after one week adaptation period at the research farm of Chattogram Veterinary and Animal Sciences University (**CVASU**), Bangladesh. The CH₄ emission were determined using a face mask linked to a 500-liter plastic tank and analysed the CH₄ concentration using a hand-held gas detector. Methane emissions in exhaled air were collected for 10 min and recorded five times a day on 2 consecutive days per period. The measurements were performed pre-feeding and then 1, 3, 6 and 9 h after feeding. The change in CH₄ concentration after breath accumulation during the day was used to compare treatments. The CH₄ concentration in exhaled air of goat fed red seaweed was 37.2% less than the control goat ($P = 0.13$). The CH₄ concentration increased after feeding and reached the highest concentration between 1 to 3 hours after feeding. In conclusion, the result of the current study suggested that red seaweed collected in the Bay of Bengal fed at 1% of the concentrate diet reduced methane emissions in goats.

34 Determination of in vitro gas production and fermentation parameters of sorghum-based feed

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Climate change has necessitated the need to incorporate less popular grains such as sorghum in livestock diets. The study was undertaken to evaluate the effect of graded sorghum inclusion on in vitro degradability (**IVDMD**), total gas production, methane and total volatile fatty acid production (**TVFA**). A total of seventeen (17) diets were formulated with sorghum to maize ratio of 100:0, 75:25, 50:50, 25:75 and 0:100. The sorghum grain used in the formulations was pretreated by soaking, malting and fermentation. Data on IVDMD, gas production, methane estimation and fermentation products were analysed using the proc GLM procedure. Inclusion rate influenced ($p < 0.05$) total gas production, methane production, Neutral Detergent Fibre (**NDF**) and Acid Detergent Fibre (**ADF**). There were no significant differences in IVDMD between the maize based and sorghum-based feed samples. Feed formulated from 100% sorghum had the highest gas (65.17ml/200mg) production and NDF (25.74%) respectively. Highest (98.14ml/g) methane production was recorded for 25% sorghum-based diets. Pretreatment methods used in the study did not significantly ($p > 0.05$) affect IVDMD, dry matter (**DM**) and crude protein (**CP**). Total volatile acid production was significantly influenced by pretreatment with feed formulated from malted sorghum grains producing 4.00mEq/g. The commercial total mixed ration (**TMR**) or control produced the lowest (2.89mEq/g) TVFA. Sorghum grain can be successfully incorporated in livestock diets to reduce methane production. Further evaluations were recommended for feed samples that produced the most favourable results. Results from the current study to be validated through in vivo studies to establish impact of feed on animal performance and methanogenic gut microbiota.

43 Effect of *Acacia mearnsii* on rumen fermentation in vivo and gas production kinetics in vitro

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The effects of types of tannin sources on ruminant remains inconsistent due to preliminary understanding of their effect on rumen fermentation. This study investigated the effect of *A. mearnsii* forage (**AMF**) inclusions in maize silage on the rumen volatile fatty acids (**VFAs**) (acetic, propionic and butyric acid); and AMF inclusions in maize silage and *A. mearnsii* tannin extract (**ATE**) in pellets on *in vitro* gas production kinetics.

To determine the VFA profile, twenty-four crossbred (Holstein-Friesian and Jersey) cows were equally assigned to four treatments; 0, 5, 15 or 25 % (as fed) AMF in maize silage in a completely randomized study design. Cows were subjected to 14 d adaptation period to treatments then 21 d of weekly rumen fluid sampling per treatment followed. Thereafter, the above-mentioned treatments were assessed for their effect on the *in vitro* gas production kinetics along with 0, 0.75, 1.5 or 3 % (as fed) ATE addition in concentrate pellets and incubated for 0, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h.

Dietary AMF inclusions did not affect ($P>0.05$) rumen volatile fatty acids but linearly increased ($P<0.05$) propionic and butyric acid. Dietary AMF did not change ($P>0.05$) the *in vitro* gas production kinetics but ATE decreased the gas production rate linearly ($P<0.05$). In conclusion, AMF and ATE slightly affected rumen fermentation since AMF inclusions increased propionic and butyric acid linearly up to the 25% inclusion while ATE inclusions decreased the gas production rate.

44 The feeding frequency of Asparagopsis oil supplementary pellets influences methane production potential in Merino sheep

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Australia has set 2050 GHG emission reduction targets. Asparagopsis, a red seaweed containing the halogenated compound bromoform, has demonstrated consistent methane abatement in total-mixed rations and grain supplementation for beef cattle and dairy cows, respectively in Australia. Australia's sheep production is most common in extensive systems, where there is a knowledge gap in understanding how an Asparagopsis-derived supplement could be administered. Supplementary feeding of pellets to manage nutritional gaps in pasture systems is common for sheep under Australian conditions and should be considered as a delivery option. The objective of this study was to assess the abatement potential of an Asparagopsis oil in supplementary pellets (Asp-pellets) when provided to sheep at differing feeding frequencies.

Seventy-two merino sheep (initial liveweight 23.3 kg $\pm$ 1.7 kg (mean $\pm$ sem); aged approximately eight months) were used in a completely randomised experimental design and fed a chaff basal diet. The following supplementary pellet treatments (200 g total per day) were tested: 1) control pellets fed every day once in the morning, 2) control pellets fed every day twice daily, 3) Asp-pellets fed every day once daily, 4) Asp-pellets fed every day twice daily, 5) Asp-pellets fed every second day once daily and 6) Asp-pellets fed every second day twice daily. The sheep were housed in individual pens at the Centre of Animal Research and Training, University of New England, Armidale, New South Wales, Australia. Animals were stratified into nine weight blocks and sheep were randomly assigned to treatments, with all treatments represented at least once per block. Control pellets were infused with 5% (by mass) canola oil and Asp-pellets were infused with 5% (by mass) Asparagopsis oil. Bromoform concentration in Asparagopsis oil was 6200 mg/kg wet matter basis. Sheep were adapted to diet for 14 days. Four $\times$ 48 h enteric methane measurement periods were conducted using eight open circuit respiration chambers, each separated by 18 days. Methane concentrations were analysed using an INNOVA 1512 fitted with a multipoint sampler. Statistical analysis was conducted in R using linear mixed effect models. Daily frequency, within-day frequency and their interaction were fitted as fixed effects, and animal and block as random effects. ANOVA was used to determine the significance ($P < 0.05$) of fixed factors and least square method was used to separate treatment means.

Methane production was influenced by daily frequency ($P < 0.001$) and within-day frequency ($P < 0.001$). In comparison to the control, sheep receiving Asp-pellets every day, twice daily, had a methane abatement of 69% and sheep feed Asp-pellets every day, once daily, had an abatement of 50%. Sheep fed Asp-pellets every second day, twice daily, had an abatement of 63% and sheep fed Asp-pellets every second day, once daily, had an abatement of 46%. Neither daily nor within-day frequency influenced total intake ($P = 0.06$ and $P = 0.94$, respectively). Feeding frequency of Asp-pellets needs to be a consideration to support tangible abatement strategies for sheep in grazing systems. For highest inhibition, pellets should be fed at least twice daily.

49 Milk from cows given a bromoform-based methane mitigant is suitable for making cheese and yoghurt

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Mitigating enteric methane emission from dairy cows is an essential part of reducing greenhouse gas emissions from the food supply chain. However, these mitigants should not adversely affect the suitability of the milk for its intended uses. We hypothesised that the milk from cows fed a bromoform-based methane mitigant would be suitable for the manufacture of yoghurt and cheese.

Ninety multiparous, Holstein-Friesian cows were allocated one of three treatments for an entire lactation; CON (0 mg bromoform/cow per day), BR1 (264 mg natural bromoform/cow per day) and BR2 (264 mg synthetic bromoform/cow per day) following a completely randomised design. Bromoform products were top dressed onto the grain dispensed to individual cows at each milking by the automatic feed system. The basal diet was 6.0 kg grain and *ad libitum* grazed pasture (predominantly perennial ryegrass, *Lolium perenne* L.) during early- and mid-lactation, and 6.2 kg grain, ~13 kg DM pasture silage, and grazed pasture during late lactation. Cows were managed as two cohorts based on calving date. Eight cows from each treatment in cohort two were randomly selected for testing of milk functionality. For acid-induced gelation tests, skimmed milk was warmed to 40°C then Glucano- δ -lactone (1.3 % w/w) added. Solution pH was measured every 5 minutes for 60 minutes. For rennet-induced gelation tests, skimmed milk was warmed to 31°C then rennet added. The onset of gelation, maximum curd firmness, and maximum firming rate were determined from firmness recorded every 15 seconds. Data were analysed using design-based analysis of variance.

In early lactation, the pH of milk in the acid gelation from the BR1 and BR2 cows at each time point were not different to that from the CON cows. However, the pH of the milk from the BR1 cows declined faster and had a lower final pH than in the milk from the BR2 cows (4.99 vs. 5.09; $P < 0.05$). In mid lactation, there was no effect of treatment on the pH of milk, except at the 30 second mark where there was a difference in milk from the BR1 and BR2 cows (6.20 vs. 6.28; $P < 0.05$). In late lactation, there was no effect of treatment on the acid-induced gelation of milk. There was no effect of treatment on the onset of rennet-induced gelation, the firmness at 60 minutes, or maximum firming rate at any stage of lactation.

Bromoform-based methane mitigants had no effect on the gelation of milk. This indicates that milk from cows treated with the bromoform-based products were suitable for use in the manufacture of yoghurt (acid-induced gelation) and cheese (rennet-induced gelation).

63 Alfalfa versus ryegrass hay: effects on methane emissions and nitrogen excretion in dairy cows

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Including legumes into ruminant diets has been proposed as a nutritional strategy to mitigate methane emissions, particularly in tropical climates with tannin-containing legumes. However, the results have been inconsistent when evaluating non-tannin legumes. This study aimed to evaluate the effects of including hay from a non-tannin legume, alfalfa, compared to ryegrass hay on methane emissions, nitrogen and energy utilization, and milk production of dairy cows in a temperate climate.

The trial was conducted at INIA's dairy farm between February and April 2021 using 8 multiparous Holstein Friesian cows in a crossover design with two 28-d periods. Cows were randomly assigned to one of two treatments: 40% diet DM of perennial ryegrass (*Lolium perenne*) hay (**PRG**), or 40% diet DM of alfalfa (*Medicago sativa*) hay (**ALF**). Diets also included grass silage (30% DM) and concentrates (30% DM), and were offered *ad libitum* once daily. During the last 7 d of each period, cows were housed individually to measure feed offered and refused, and milk production and composition. Enteric methane was measured using the sulphur hexafluoride technique, and total faeces and urine were collected to assess nutrient utilisation. Results were analysed based on the fixed effects of the treatments, and the random effect of period and cow.

The ALF diet had more CP (20.9 vs. 17.5% DM) and less NDF (45.8 vs. 50.4% DM) than PRG. Compared to PRG, ALF increased DMI (15.6 vs. 17.1 kg DM/d; $P = 0.027$) and CP intake (2.72 vs. 3.58 kg DM/d; $P < 0.001$). Apparent digestibility of CP was 9.0% higher with ALF than PRG ($P = 0.036$). Total methane emissions tended to increase by 19.4% with ALF (432 vs. 477 g/d; $P = 0.076$), although methane yield (g CH₄/kg DMI), intensity (g CH₄/kg milk yield) and Y_m (MJ CH₄/100 MJ GE intake) were unaffected ($P \geq 0.54$). Cows fed ALF tended to increase milk yield (+1.3 kg/d, $P = 0.10$) and gained more body mass (+0.62 kg/d; $P = 0.02$). Milk composition was unaffected by treatments, except for an 11 % increase in milk urea content with ALF ($P = 0.006$). Compared to PRG, the ALF diet shifted N excretion towards urine (159 vs. 242 g/d; $P = 0.046$).

In conclusion, alfalfa hay inclusion tended to increase absolute methane emissions and did not decrease enteric methane yield or intensity. While it improved milk production, it also led to higher N excretion in faeces and urine, highlighting a trade-off between productivity and environmental impact. In agreement with previous results, a non-tannin containing legume did not mitigate enteric methane emissions.

65 Assessing IPCC Tier 2 Methodology for Enteric Methane Estimation in Tanzanian Rangeland Cattle: Evidence of Systematic Underestimation

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Livestock contributes over 30% of global methane (**CH₄**) emissions, a potent greenhouse gas (**GHG**) that must be mitigated to combat climate change. Accurate estimation of these emissions is crucial for national GHG inventories and mitigation strategies. The Intergovernmental Panel on Climate Change (**IPCC**) Tier 2 methodology is the recommended approach for estimating enteric CH₄ emissions, as it accounts for local livestock characteristics. However, its equations are primarily derived from data from temperate regions, raising concerns about their accuracy in African livestock systems. This study evaluates the accuracy of the IPCC Tier 2 methodology for estimating dry matter intake (**DMI**) and daily methane production (**DMP**; g CH₄/head/day) in Tanzanian Short Horn Zebu (**TSHZ**) and Boran cattle, using five dietary treatments: grazing only (GrazC00), grazing plus 50% ad libitum concentrate (GrazC50), and hay plus 60% (HayC60), 80% (HayC80), or 100% (HayC100) ad libitum concentrate. Two estimation methods were compared: the IPCC Tier 2 default approach (**Tier2IPCC**) and a modified approach incorporating measured DMI (**Tier2intake**). Measured DMI was 44% higher than Tier2IPCC estimates (7.5 vs. 5.2 kg/day). Consequently, Tier2intake estimated significantly greater DMP than Tier2IPCC (120 vs. 80 g CH₄/head/day for TSHZ; 148 vs. 107 g CH₄/head/day for Boran; $p < 0.05$). These trends were similar across the five dietary treatments in the two breeds. These findings highlight potential systematic underestimation of CH₄ emissions by Tier2IPCC for other cattle, which could impact national GHG inventories. Refining the IPCC Tier 2 methodology to better reflect intake variations in African livestock systems is essential for improving emission estimates and informing mitigation strategies.

68 Seasonal variation in nutraceutical properties of *Searsia* species found in North West province, South Africa

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The study was carried out to assess the seasonal variation in the chemical composition, qualitative and quantitative phytochemicals and in vitro dry matter degradability (IVDMD) of *Searsia* species. *Searsia lancea* and *Searsia pyroides* leaves were harvested on the North-West University experimental farm in the North West Province of South Africa in the autumn, winter, spring, and summer seasons. The samples were analysed for chemical composition, quantitative phytochemicals, and IVDMD. Solvents (methanol and distilled water) were used to prepare cold extracts for qualitative phytochemicals. For IVDMD, the rumen fluid was collected from a fistulated Bonsmara cow. Data were analysed using a two-way analysis of variance (SAS). The lowest ($P < 0.05$) neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and cellulose were recorded for *S. lancea* compared to *S. pyroides*. The highest ($P < 0.05$) crude protein (CP) content ($P < 0.05$) was produced in summer. The lowest ($P < 0.05$) NDF, ADF, ADL, and cellulose ($P < 0.05$) was in the summer season, compared to other seasons. Within each species, both species had the lowest ($P < 0.05$) NDF and ADL in summer. There was no variation ($P > 0.05$) in the content of ADL, cellulose, and hemicellulose between species in each season. Saponins were highly present in all seasons of both extracts in *S. lancea*, while they were moderately present in *S. pyroides*. Phlobatannins were moderately present in the methanol extract, while absent in the water extract in all seasons. *Searsia pyroides* had the lowest ($P < 0.05$) total phenolic, total tannins and condensed tannins (CT) content ($P < 0.05$) compared to *S. lancea*. The highest ($P < 0.05$) IVDMD ($P < 0.05$) was recorded for *S. pyroides* at 36 and 48 h in all seasons. Both species have potential to be used as feed due to high CP and low fibre. However, in all seasons, these species had the highest CT concentration, which can reduce their feeding value when CTs are not deactivated.

73 Effect of drinking water application of 3-nitrooxypropanol on performance and gaseous emissions in dairy cows

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The feed additive 3-nitrooxypropanol (**3-NOP**) reduces CH₄ emission of dairy cows by an average of 30%, particularly when included in a total mixed ration, resulting in regular intake of 3-NOP throughout the day. On pasture however, 3-NOP is consumed intermittently by cows, only during feed supplementation times. As CH₄ levels of 3-NOP treated animals return to control levels within 3 h post-supplementation, intermittent intake of 3-NOP results in a lower reduction of CH₄ emission compared to regular intake of 3-NOP. Administering 3-NOP through drinking water to grazing dairy cows might offer a solution. This study aimed to investigate the effects of administering 3-NOP through drinking water on (1) dry matter and water intake, lactation performance, and gaseous emissions in dairy cows, and on (2) the diurnal pattern of CH₄ and H₂ emissions. A key focus was the synchronization between feed intake, 3-NOP intake via drinking water, and CH₄ emission, compared to regular intake of 3-NOP in a total mixed ration. A double 4 × 4 Latin square design was used with 8 lactating dairy cows and 4 treatments: a negative control without 3-NOP supplementation (**CON**), a positive control with 60 mg 3-NOP/kg DM in a total mixed ration (**TMR60**), 3-NOP via drinking water at a target dose equivalent to 60 mg 3-NOP/kg DM as provided via feed (**WATER60**), and 3-NOP via drinking water at a target dose equivalent to 90 mg 3-NOP/kg DM as provided via feed (**WATER90**). The basal diet was identical for all treatments and consisted of grass silage, corn silage, and concentrate, except that the TMR concentrate contained 3-NOP. Treatment periods lasted 3 weeks, with 2 weeks of adaptation in tie-stalls followed by 1 week of measurements in climate respiration chambers. Cows had ad libitum access to feed and drinking water (i.e., with or without 3-NOP, depending on the treatment). Data were analysed using the MIXED procedure in SAS, with treatment and square as fixed effects, and cow within square and period as random effects. Diurnal variables (e.g., gaseous emissions and water uptake) were subjected to repeated measures ANOVA to account for repeated sampling within cows, with treatment, square, and time as fixed effects, and cow within square and period as random effects. We hypothesized that administering 3-NOP through drinking water would mitigate CH₄ emission with a reduction potential similar to that in TMR-cows, without negatively impacting dry matter intake and lactation performance. The results are lacking, because the study is currently ongoing. We confirm that the study will be completed and shared at the time of the conference.

91 Nano ZnO-modified sugarcane biochar in acacia diets: ruminal fermentation, digestibility and methane emission in vitro

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Modified biochar may have the potential to reduce greenhouse gas emissions while enhancing ruminal nutrient degradation in *Acacia saligna*-based diets. In this study, sugarcane bagasse biochar was modified with green-synthesized nano-zinc oxide (**SC-ZnO**) and evaluated for its physicochemical properties. We used ZnO as it is known for its potential to reduce methane production by modulating rumen microbial activity and shifting fermentation pathways away from hydrogenotrophic methanogenesis. The biological efficacy of SC-ZnO was assessed through an *in vitro* gas production assay; three buffalo calves from the slaughterhouse of Alexandria University were used as ruminal fluid donors per each incubation run (n=3). The experimental treatments were: the clover Berseem hay basal diet (60% clover to 40 % concentrate without any supplementations), the Acacia diet (30% acacia leaves, 30% clover, 40% concentrate), the Acacia diet supplemented with unmodified sugarcane bagasse biochar (U-SC) at 2.5 g/kg DM, two SC-ZnO diets that consisting of Acacia diet supplemented with SC-ZnO at 0.5 (low) and 1 (high) g/kg DM. The incubation diets contained similar amounts of organic matter to ensure comparability of degradability results across treatments. The results of the particle size distribution analysis showed a successful transformation from a broad distribution, ranging from 4.5 to 64.6 µm for U-SC to a narrower distribution in the nanoscale range, ranging from 44 to 71 nm for SC-ZnO. Zinc oxide only appeared at 6.13±0.32 % for the SC-ZnO while it was absent in U-SC. The modification of biochar resulted in numerical increases in the values of the cation exchange capacity, specific surface area, and negative Zeta potential compared with the U-SC. The results were statistically analyzed using ANOVA with SAS software to assess the significance of treatment effects on methane emissions and ruminal fermentation parameters with 4 technical replicates x 3 biological replicates (incubation runs)/treatment. The results of the *in vitro* assay indicated that the Berseem clover diet exhibited higher (P<0.05) total gas production (**GP**) and methane CH₄ values than Acacia diets supplemented or not with SC-ZnO_{low}. Significant reductions (P<0.05) in CH₄ and CO₂ emissions were observed among the Acacia diets; however, the Acacia diet supplemented with ZnO showed a lower reduction in CO₂ production compared to the unsupplemented Acacia diet, indicating a more selective mitigation of methane rather than a general suppression of fermentation. Among the experimental treatments, the Berseem clover diet and Acacia diet supplemented with SC-ZnO_{low} had similarly high (P<0.01) organic matter degradability (**TDOM**). The Acacia diet without any supplements and those supplemented with the high dose of SC-ZnO had the lowest (P=0.0025) total short-chain fatty acids (**SCFA**) contents, while Acacia supplemented with U-SC or SC-ZnO at a low dose had the highest (P=0.0025) total SCFA values. Similar significant increases (P=0.0025) in total SCFA were observed in Berseem clover hay and all Acacia diets with feed additives. The current results confirm that SC-ZnO supplemented at a low level can indeed exert beneficial effects on modulating ruminal fermentation patterns, resulting in reduced CH₄ production without adverse effects on nutrient degradability.

94 Modified nano bentonite clays with *Saccharomyces cerevisiae* reduce methane emissions and enhance ruminal fermentation efficiency

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Modified nano clays, synthesized via ion-exchange reactions, offer a promising strategy for reducing greenhouse gas (**GHG**) emissions in ruminant systems. Combining these clays with *Saccharomyces cerevisiae* may enhance GHG mitigation and improve ruminal nutrient degradability compared to ionophore antibiotics. In this study, a modified nano bentonite (**MNB**) was developed by organically modifying natural bentonite with sodium dodecyl sulfate (**SDS**). The physicochemical properties of MNB were characterized and compared with natural bentonite (**NB**). *In vitro* gas production techniques were used to evaluate the biological activity of these clays. Treatments included a control (basal diet without additives), sodium monensin (40 mg/kg DM), yeast (*S. cerevisiae*) at $2 \times 10^8 \times 10^8$ CFU/g DM, NB (5 g/kg DM), and MNB with or without yeast at low (0.5 g/kg DM) and high (1 g/kg DM) doses. Statistical analysis was conducted using ANOVA in SAS, with the incubation bottle as the experimental unit. MNB showed a significant reduction in particle size (72 nm) compared to NB (63,230 nm). Additionally, MNB exhibited higher specific surface area, electrical conductivity, and cation exchange capacity than NB. Sulfur (0.15 ± 0.03 atomic %) and sulfur-containing functional groups at 639.06 cm^{-1} and 507.93 cm^{-1} were detected exclusively in MNB. Supplementation with MNB and yeast significantly increased ($P < 0.01$) gas production compared to the monensin treatment. Furthermore, MNB combined with yeast at both low and high doses significantly reduced ($P < 0.001$) methane (**CH₄**) production compared to control and monensin treatments, measured by various metrics such as (ml/g degraded cellulose and organic matter) and (ml/g degraded hemicellulose). The reduction in CH₄ was combined with significant increase in hydrogen sulfide (**H₂S**) production compared to the control and monensin treatments ($P < 0.05$). MNB combined with yeast at high dose had positive effects ($P < 0.05$) on degraded hemicelluloses compared with other treatments. Yeast supplementation influenced protozoal populations ($P < 0.05$), with higher counts observed in some species (e.g. *Epidinium spp.*) compared to control and monensin treatments. Ammonia levels did not differ significantly among treatments, while yeast supplementation resulted in higher acetate percentages but lower propionate and butyrate percentages compared to control and monensin treatments ($P < 0.05$). Conversely, MNB supplementation with yeast, led to reduced ($P < 0.05$) acetate percentages but increased ($P < 0.05$) propionate and butyrate percentages, suggesting differential effects on SCFA profile and ruminal fermentation pathways. These findings suggest potential modulatory effects of yeast and MNB on rumen fermentation dynamics and microbial populations.

99 Bromoform-based Investigational Veterinary Product fed to lactating dairy cows shows no adverse effects on behaviour

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Mitigating enteric methane emissions from ruminants is a critical research focus to improve the environmental sustainability of livestock production. As society increasingly demands that animal welfare be incorporated into sustainable practices, understanding the welfare implications of new technologies is also essential. An Investigational Veterinary Product (IVP) containing synthetic bromoform has recently emerged as a promising option for consistently reducing methane emissions from ruminants. However, the potential effects of including the IVP on eating, rumination and locomotion behaviour (critical animal welfare indicators) remain unclear. This study assessed the response of feeding an IVP containing bromoform at two rates to lactating dairy cows as a supplemental feed offered twice daily at milking on i) eating and rumination behaviour; and ii) locomotion behaviour. Thirty multiparous, Holstein-Friesian cows (214 ± 15.2 days in milk) were allocated one of the three treatments; CON (0 mg bromoform/cow per day), LowBR (227 mg bromoform/cow per day) and HighBR (455 mg bromoform/cow per day) following a completely randomised design. The IVP was provided by Rumin8 (Rumin8 Pty Ltd, Perth, Australia), premixed with the grain and offered in the dairy during each milking. The basal diet consisted of vetch hay *ad libitum* and 3.1 kg dry matter of a grain mix fed in the dairy per milking. Vetch hay was fed across thirty stalled load cell-equipped bins, with daily cow intake measured electronically by linking bin weights to each cow's electronic identification (Gallagher Animal Management Systems, New Zealand). Following adaptation to dietary treatments, cows were equipped with a noseband pressure sensor and a pedometer from the RumiWatchSystem (Itin+Hoch GmbH, Liestal, Switzerland) to assess eating, ruminating, and locomotion behaviour for 11 days. Data were processed with the RumiWatch Manager (version 2.1.0.0, Itin+Hoch GmbH, Liestal, Switzerland) and each response variable were analysed by ANCOVA with milk yield, liveweight, dry matter intake, and parity as covariates because treatment groups were balanced for these in the design. This study showed no significant effect of the IVP on dry matter intake ($P=0.841$), eating ($P=0.131$) and rumination behaviour ($P=0.762$). There was no effect on the circadian pattern of eating ($P=0.251$) and rumination behaviour ($P=0.291$). Feeding the IVP did not impact lying ($P=0.894$) or standing behaviour ($P=0.909$). Although cows fed the IVP showed a slight increase in walking duration ($P=0.005$), the difference was negligible from a practical perspective given it equated to only six minutes per day. These findings indicate that feeding dairy cows up to 455 mg of bromoform/cow per day in the IVP does not change their eating, ruminating, and locomotion behaviour, addressing concerns about potential stress or discomfort to the animal. These results suggest that the overall health and well-being of the animals remained unaffected, as animal behaviour is a reliable indicator of these factors. Thus, we conclude that the bromoform-based IVP might be a potential technology to reduce enteric methane emissions without compromising animal welfare.

109 Environmental impact of the EcoFeed® Index: Reducing greenhouse gas emissions in the dairy sector

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Ecofeed® is a genetic index designed to identify cattle with superior feed efficiency based on residual feed intake (**RFI**) information from female progeny. This study aimed to evaluate the effectiveness of the EcoFeed® index in reducing the carbon footprint (measured in kg of CO₂ equivalent [**eq.**]) of Holstein females based on genetic selection for RFI. A dataset containing 1,608 Holstein females with genomic breeding values for RFI (**gRFI**), as reported by STgenetics using the Ecofeed® index, was used in this study. Animal performance and feed intake records were collected for each animal during both the heifer and lactating cow stages at the STgenetics Ohio Heifer Center (South Charleston, OH, USA). Animals were divided into three classes: low gRFI, average gRFI and high gRFI based on +/- ½ standard deviation from the mean gRFI. Carbon footprint was determined using Life Cycle Assessment (**LCA**) framework, incorporating feed input emissions and IPCC (2019) models for enteric methane and manure emissions. Management practices, including stage-specific rations and bedded-pack manure management, were also factored into the analysis. As expected, low gRFI females consumed 10% less feed per unit of energy-corrected milk (**ECM**) compared to their high gRFI counterparts (0.79 vs 0.87 kg feed/kgECM, respectively) with no difference in ECM production. The carbon footprint per kg of ECM was 1.04, 1.00, and 0.97 kg CO₂ eq. for high, medium, and low gRFI classes, respectively. Enteric methane had the greatest contribution to total emissions (47%) followed by feed production (21%), manure (20%), and electricity use (13%). Over an average lifetime milk production of 30,642 kg ECM, low gRFI females consumed 2,451 kg less feed and produced 2,326 kg less CO₂eq. compared to high gRFI females. These findings demonstrate that genomic selection for RFI through the EcoFeed® index is a potent strategy to enhance both economic and environmental sustainability in the dairy industry.

119 Impact of effective microorganisms-treated wheat bran on nutritional parameters and methane production

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The present study investigates the effects of Effective Microorganisms-treated wheat bran (**EMWB**) on organic matter digestibility (**OMD**), metabolizable energy (**ME**), short-chain fatty acids (**SCFA**) production, and in vitro gas and methane production in a mixed ruminant diet. The diet comprised 70% native pasture hay and 30% concentrate mix, with the latter including varying levels (0%, 33%, 66%, and 100%) of EMWB corresponding to EM0, EM33, EM66 and EM100, respectively. The total gas production at all incubation hours (3, 6, 12, 24, 48, 72 and 96 hr.) were higher ($p < 0.05$) for EM100 and followed by EM66. The highest ($p < 0.05$) "a" fraction of gas production value was recorded for EM0 (1.56). The "b" fraction value was high ($p < 0.05$) for EM100 (33.8) = EM66 (32.6) and low for EM33 (29.9) = EM0 (28.6). Gas production potential (a+b) value was greater ($p < 0.05$) for EM100 (34.2) = EM66 (32.8) and lower for EM0 (30.2) = EM33 (30.4). The rate constant of gas production © was greatest ($p < 0.05$) for EM100 (0.25) and lowest for EM0 (0.08) = EM33 (0.11). OMD and ME were in order of EM100 (56.6, 7.60) > EM66 (54.2, 7.14) > EM33 (50.1, 6.49) = EM0 (48.1, 6.21) respectively. The value of SCFA was in order of EM100 (0.77) = EM66 (0.72) > EM33 (0.60) = EM0 (0.57). Methane (**CH₄**) production at 24 hours showed a decreasing trend with increasing EMWB levels, underscoring the potential of EMWB as a sustainable feed supplement that not only enhances OMD and SCFA production but also contributes to reducing CH₄ emissions from livestock.

125 Eco-friendly feed formulation is an efficient way to reduce climate change impact in pig production

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Animal feeding accounts for 70% of climate change impacts (**CC**), 70% of energy use (**NRE**), 100% of land occupation (**LO**), 60% for eutrophication (**EU**) and 30% for acidification (**AC**). These large contributions are due mainly to the processes used to grow crops for feed ingredients (use fertilizers and pesticides, land occupation and land transformation, energy consume and large-scale transportation network). Feed ingredients can vary in their environmental impacts; certain ingredients, like imported soybean meal, are resource-intensive compared to alternative protein sources that can be locally produced (peas, fababeans, or high-protein agricultural co-products). Therefore, there is a possibility to reduce environmental impacts by selecting feed ingredients with relatively low impacts. To this end, Garcia-Launay et al. (2018) developed a multi-objective (**MO**) formulation method based on the environmental impacts of feed ingredients (CC, NRE, LO, EU and AC). The aim of this study was to assess the effects of such environmentally friendly pig diets on nutrient digestibility, energy utilization, performance of finishing pigs and environmental impacts calculated at farm gate. The Regional Ethical Committee (authorization no. 2019041815163846) approved the experiment. Two diets were compared: a control diet based on typical practices in French commercial farms (Control-diet) and an eco-friendly diet formulated using the MO method (Eco-diet). Twelve Pietrain x (Large White x Landrace) entire males (81 kg) were used, with six pigs per diet group. The pigs were housed individually in digestible cages, equipped to measure individual feed and water intake, feeding behaviour, and allow for separate collection of feces and urine. Following an adaptation period of 14 days, the pigs were housed in climate-controlled respiration chambers (one pig per chamber) for six days to measure nutrient digestibility and energy utilization, as outlined by Labussière et al. (2013). We then tested the experimental diets on the performance of finishing pigs from 60 to 115kg. Sixty-two pigs (Pietrain x (Large White x Landrace) entire males) were fattened in a single pen equipped with an automatic weighting device and with automatic feeders, allowing daily individual recording of live weight and feed consumption. Pigs fed one of the two experimental diets, feed and water were provided ad libitum. Carcass weight, lean meat percentage were measured at slaughterhouse. We calculated the environmental impacts of multiobjective formulation at farm gate. LCA was conducted individually for each pig according to individual animal performance and impacts of diets. Experimental datas were subjected to an analysis of variance that tested the effects of diet (R software). Results were considered significant for P-values lower than 0.05. At feed level, per kg of feed, the Eco-diet in comparison with the Control-diet reduced CC by 24%, NRE by 10%, AC by 18%, EU by 9.5% and LO by 1%. No significant differences were observed between the dietary treatments in terms of growth performance, including feed intake, average daily gain, feed conversion ratio, or water consumption in the two experimental studies. Using feed ingredients with lower environmental impacts is an efficient way to reduce impacts of pig production without negative consequences on animal performance.

143 Inclusion of forage chicory to reduce methane emissions from beef cattle: an *in vitro* study

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Chicory (*Cichorium intybus* L.) is a high-quality perennial forb-based forage containing secondary metabolites, including tannin, and can potentially improve forage quality and reduce enteric methane (**CH₄**) emissions in beef cattle. It can serve as an alternative non-traditional forage for summer grazing pasture in western Canada. Therefore, the objective of this study was to evaluate the degradability and fermentation characteristics as well as enteric CH₄ mitigation potential of forage chicory with or without cool-season grass and/or legume using the *in vitro* batch culture technique. Whole plant Choice chicory (*Cichorium intybus*; **C**), AAC Glenview sainfoin (*Onobrychis viciifolia*; **S**), or Fleet meadow brome grass (*Bromopsis biebersteinii*; **G**) were harvested from two field plot replications over two years in Swift Current, Saskatchewan, Canada. The samples were combined to form a single, two, or three-species mix for each field block and year. The forage treatments included: C (100:0:0), S (100:0:0), G (100:0:0), CS (50:50:0), CG (50:50:0), SG (50:50:0), and CSG (33.3:33.3:33.3). Samples were incubated for 48-hour with three runs and two replicates per run. Data were analyzed using the “nlme” package of R (version 4.3.2) with a model that included the fixed effect of forage treatments ($n = 7$) and the random effects of run ($n = 3$), year ($n = 2$), and field plot ($n = 2$). Neutral detergent fibre (**NDF**) content on a dry matter (**DM**) basis was higher ($P < 0.001$) for the G treatment (62.3%) and lower for the S (38.0%) and the C (31.4%) treatments. As a result, the NDF content of the CS mix (38.4%) was lower ($P < 0.001$) as compared to the mixtures containing grass (ranging from 45.4% to 49.9%). The NDF degradability (g kg⁻¹NDF) for the CG mix (554) was similar ($P \geq 0.95$) to that of C (494) and G (514) but higher ($P < 0.001$) than S (322) and the other forage mixes (396 to 479). The lower NDF in chicory coupled with its higher degradation increased ($P \leq 0.01$) the *in vitro*DM true degradability (g kg⁻¹DM) for C treatment (820) as compared to S (745), G (698) and the SG mix (742). Consequently, total volatile fatty acid concentration was higher ($P \leq 0.04$) for both C (84.7 mM) and CG mix (84.5 mM) relative to S (82.9 mM) but was similar ($P \geq 0.15$) to G, the two- or three-mix forage treatments, ranging from 83.3 to 83.5 mM. Furthermore, except for CS (4.5 mL), CH₄ production was significantly lower ($P < 0.001$) for C (4.06 mL) as compared to S, G, and their mixtures (4.71 mL to 5.32 mL). However, CH₄ yield (mg/g degradable DM) was lowest ($P < 0.001$) for C (7.4) and highest ($P \leq 0.04$) for G (11.5), but similar ($P \geq 0.36$) among S and the other forage-mix treatments ranging from 8.8 to 11.5. In summary, the results suggest that the use of chicory with or without cool season grass and/or legume may improve forage quality and degradability and decrease enteric CH₄ production.

155 Productivity of feedlot cattle improved with methane inhibition by *Asparagopsis* bioactives stabilized in canola oil

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Research using open-circuit respiration chambers has established that *Asparagopsis* bioactive compounds stabilized in canola oil (**Asp-Oil**), delivering 34 mg bromoform (**CHBr₃**)/kg dry matter intake (**DMI**), inhibits methane (**CH₄**) emissions > 98% in feedlot cattle. In this study (approved by the University of New England Animal Ethics Committee ARA22-046), Asp-Oil was fed at 35 mg CHBr₃/kg DMI in a beef feedlot finisher diet under highly replicated and commercially relevant conditions, and adequately powered to confirm differences as low as 3.2% in feed conversion efficiency (**FCE**). The study also evaluated effect of Asp-Oil on carcass and meat-eating qualities, animal health, and residues. The experimental design consisted of 300 Angus-Shorthorn (*Bos taurus*) steers in 30 pens of 10 head and fed a barley-based ration, representative of Australian short-fed feedlot diets. The steers were supplemented with canola oil (Control, n = 15), or Asp-Oil (n = 15) for total 81-d, inclusive of 21-d transition to full Asp-Oil and grain inclusion in the finisher ration.

The Asp-Oil cattle presented 67% of CH₄ emissions measurements below the GEM's limit of detection thereby confounding direct CH₄ accounting, however an inhibition range of 58-98% was demonstrated by collectively using GEM and RC measurements. The Asp-Oil induced FCE improvements of 7.4% in the finisher diet ($P = 0.018$), and 5.6% for the full collective of transition and finisher periods ($P = 0.004$). During the transition steps the steers had not yet received their full allocations of grain and Asp-Oil which is responsible for the marginally lower FCE benefits prior to stabilization on the finisher diet. A 4.1% lower DMI in the finisher period ($P = 0.022$) contributed to benefits in FCE with no effect on daily weight gains. Feed expenses and cost of weight gain reduced \$0.35/head/day and \$0.23/kg ($P = 0.007$), respectively, in steers receiving Asp-Oil. Residues of *Asparagopsis* CHBr₃ were not detected in any food product and only trace iodide and bromide were detected in livers and kidneys of both Control and Asp-Oil steers and at levels safe for human consumption. Steers demonstrated normal rumen development typical of feedlot style diets. Consumer eating quality scores for tenderness, juiciness, flavour, overall liking, and MQ4 were not affected by Asp-Oil ($P > 0.05$).

This research has been able to confirm, with high levels of replication and statistical power, that productivity gains are expected with high levels of CH₄ inhibition by feeding a TMR delivering *Asparagopsis* bioactives prescribed as 35 mg CHBr₃/kg DMI in commercial feedlots. Efficiency gains as FCE were achieved by a reduction in feed DMI, with no significant change in ADWG, HSCW, or carcass grading attributes, resulting in a lower cost of production. Furthermore, Asp-Oil was confirmed to have no effect on food quality and safety, or animal welfare.

157 Evaluating oral swabbing as a practical alternative to rumen sampling for large-scale methane emission studies in grazing animals

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As an integral aspect of their digestive process, ruminants, including cattle, generate greenhouse gases (**GHGs**), notably methane. In 2022, livestock farming contributed to a substantial 11.1–19.6% of total GHG emissions, solidifying its position as a significant anthropogenic source of GHGs. Enteric methane, a prevalent by-product of livestock farming, significantly contributes to global warming. The escalating global population, coupled with an increasing demand for protein-based diets, anticipates the continued expansion of livestock ranching, intensifying concerns about the adverse environmental impact caused by methane emissions.

Within the digestive system of ruminants, the first compartment of their specialized stomach, called the rumen, contains a consortium of commensal microorganisms. These microorganisms facilitate the breakdown of complex carbohydrates through the enteric fermentation process. Methanogenic microbes harness the hydrogen produced during fermentation to reduce carbon dioxide, which eventually yields methane as a by-product. Several studies have predicted methane emission from ruminant microbiomes, however the widespread application of the method is limited due in part to sampling difficulty and animal welfare concerns. Common methods, such as ruminal fistulation, stomach tubing, or rumenocentesis, are invasive and contrary to animal welfare principles. Moreover, these methods are labour-intensive, increasing the time cattle must remain restrained in the crush and elevating their stress levels. As a result, rumen collection is impractical and unsuitable for large-scale deployment or commercial farm settings.

The process of rumination, where ruminants regurgitate cud from the rumen to the mouth, suggests a potential link between the oral and rumen microbiomes in relation to the methane emission phenotype. In this study, 250 cattle oral microbiome samples were sequenced and analysed. Taxonomic and gene functional abundance matrices were generated to build predictive models for estimating methane emission phenotypes based on methane output (grams per day) measured using the GreenFeed system. Microbiabilities (m^2) - the proportion of phenotypic variation explained by the microbiome relationship matrix—were assessed using both taxonomic (m^2 , 0.727) and functional (m^2 , 0.696) annotations. Correlations between predicted phenotypes and GreenFeed-measured phenotypes will be evaluated using the BLUP model's predictive microbial ability in multiple-fold cross-validations, and the complete results will be reported during GGAA2025. This study demonstrates the potential of employing cattle oral metagenomic data as a proxy for methane emission phenotypes, offering a non-invasive, efficient alternative to rumen sampling, particularly in large-scale commercial grazing herds.

160 Insect based poultry feed integration with conventional diet significantly reduces potent greenhouse gases emission

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There is growing concern about meeting the increasing demand for animal products with a low environmental footprint while preserving livestock's economic, social, and cultural benefits. While much research focuses on mitigating greenhouse gas (GHG) emissions from large livestock, the role of poultry in GHG emissions and strategies to reduce these emissions receive less attention. We hypothesized that feed composition and quality significantly influence chicken productivity and environmental sustainability. This study examined how full-fat and defatted black soldier fly larvae (BSFL)–based poultry diets affect GHG emissions and secondary metabolite profiles of chicken poop, a major GHGs source. We demonstrate that conventional and insect-derived diets differ in GHGs emissions. Feed derived from black soldier fly larvae produced higher nitrous oxide (N₂O), soybean and insect derived feeds yielded equivalent methane (CH₄) emissions. Fish meal resulted in lower emissions of both N₂O and CH₄. Notably, the incorporation of insect-derived protein with soybeans, or fish meal significantly reduced potent greenhouse gases, CH₄ and N₂O emission. Regarding carbon dioxide (CO₂), defatted BSFL and soybean-based diets generated minimal emissions compared to full-fat BSFL and fish meal. However, mixing BSFL with conventional feeds slightly increased CO₂ emissions. Gut secondary metabolite specifically alcohol, ester and acids, negatively correlated with CH₄, N₂O and CO₂ emissions, respectively suggesting they may have contributed to the variation in GHGs emissions. Integrating insect derived protein with conventional chicken diet significantly reduced potent greenhouse gases emissions without compromising chicken biological and economic fitness offering a sustainable strategy for mitigating environmental impacts in poultry production.

162 Assessing carbon footprint, feed quality, and value chain dynamics in Ethiopia's Assela & Jimma milk sheds.

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The dairy sector is a significant contributor to global greenhouse gas (**GHG**) emissions, with feed production and nutrition playing a crucial role in these emissions. This study investigates the role of dairy feed value chain actors, the chemical composition of feed, and the associated carbon footprint in the Assela and Jimma milk sheds. The study employed a multistage sampling technique to survey 246 dairy producers and key feed value chain stakeholders. Data were systematically gathered through household surveys, key informant interviews, and laboratory analyses. The study estimated greenhouse gas emissions from dairy feed production using a Life Cycle Assessment (**LCA**) approach focused on on-farm feed production and transportation, applying IPCC guidelines and statistical evaluations performed using SPSS. The dairy feed value chain in both milk sheds comprised input suppliers, feed producers, processors, traders, and end users. Feed marketing channels in Assela and Jimma milk sheds were simple, with higher Gross Marketing Margins (**GMM**) in Assela due to direct producer-to-consumer sales, while Jimma's GMM was lower due to traders charging higher prices compared to cooperatives. The highest Gross Marketing Margin for wheat bran and concentrate feed was recorded in Assela, specifically in the direct producer-to-end-use channel (100%). In terms of feed composition, Assela exhibited the highest crude protein content in both roughage and concentrate, whereas Jimma had higher crude fiber content. Assela milk shed has higher emissions due to higher fertilizer use, machinery, and feed processing/transportation compared to Jimma milk shed. However, both milk sheds contributed a very small percentage to Ethiopia's overall GHG emissions, with Assela emitting 0.0000057 tons of CO₂ and Jimma emitting 0.0000029 tons. Despite these relatively small contributions, improving fertilizer practices and reducing reliance on synthetic fertilizers could help reduce emissions further. The analysis clearly demonstrated an inverse correlation between feed productivity and CO₂ emissions across both milk sheds, highlighting the potential for improved feed production to mitigate environmental impacts. In Assela, a 1-quintal increase in feed production corresponded to a 7% reduction in CO₂ emissions, whereas Jimma showed a more modest 1% decrease. Moreover, the transportation of feed emerged as a significant factor contributing to CO₂ emissions, with Jimma experiencing a 44% increase in emissions per quintal, while Assela faced a 10% rise. These discrepancies were primarily attributed to the overreliance on synthetic fertilizers, particularly in Assela, and the long-distance transportation of feed in Jimma. The findings underscore the critical need to integrate organic fertilizers with synthetic alternatives to optimize soil fertility and reduce emissions in Assela, while promoting localized feed processing in Jimma to decrease transportation-related emissions. Furthermore, the data suggest that enhancing feed productivity and employing climate-smart agricultural practices can lead to substantial reductions in the carbon footprint of dairy systems. The study also emphasizes that strategic interventions in fertilizer management and feed logistics are crucial for mitigating the greenhouse gas emissions associated with dairy production in Ethiopia. Given that dairy systems are central to Ethiopia's agricultural economy, these improvements could significantly contribute to the country's broader climate change mitigation goals.

176 Effects of *Asparagopsis*-based Feed Additive on Rumen Fermentation, Growth Performance, and Methane Production in Hanwoo

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Macroalgae has shown significant potential in inhibiting enteric methane (**CH₄**) emissions when included as a feed additive in the ruminant's diet. Among them, the red seaweed *Asparagopsis* sp., which is rich in natural halogenated compounds such as bromoform, has been demonstrated to have anti-methanogenic properties and can reduce CH₄. Although *Asparagopsis* has demonstrated potential, its dietary use in Korean native cattle (Hanwoo) remains underexplored. Therefore, this study aimed to investigate the effects of a formulated seaweed-based supplement on rumen fermentation, growth performance, and methane emissions in Hanwoo steers fed a high concentrate diet. Twelve Hanwoo steers with an average body weight of 625.92 ± 64.14 kg and approximately 21 months old upon experiment onset were assigned in a replicated 3 × 3 Latin square design with three treatments and three periods. The steers were randomly allocated to three groups that were offered basal diet (9.6 kg of commercial concentrate feed and 2.4 kg of forage) that varied only in the addition of the respective feed additives: no additive (Control), 0.5% inclusion level of *Asparagopsis*-based feed additive (**AS**; CH4 Global, Inc.) with bromoform content of 4 mg/g dry matter (**DM**) (**AS4**), and 0.5% inclusion level of AS with bromoform content of 7 mg/g DM (**AS7**). The study lasted for 97 days and consisted of a 20-day diet adaptation phase and three 21-day experimental periods that were separated by two 7-day washout periods. Rumen fluid was collected using an oral stomach tube at the end of each period for pH, ammonia-nitrogen, and volatile fatty acid (VFA) analysis. Total weight gain, average daily gain (**ADG**), and feed efficiency (**FE**) were calculated. Enteric methane emissions were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD). Methane emission was expressed as CH₄ production (g/d), yield (g/kg dry matter intake; **DMI**), and intensity (g/kg BW^{0.75}). Data were analyzed using the general linear model (**GLM**) procedure of SAS ver. 9.4. Tukey's honestly significant difference (**HSD**) test was used to identify differences between treatment groups. Statistical significance among treatments was declared at $P < 0.05$. The results showed that the supplementation of *Asparagopsis*-based feed additive in steers did not alter the rumen pH, ammonia-nitrogen concentration, and VFA ($P > 0.05$). In addition, the supplementation of *Asparagopsis*-based feed additive did not adversely affect ($P > 0.05$) the growth rate and feed efficiency of steers. Methane production in steers was significantly reduced by 37.25% and 45.88% in AS4 and AS7, respectively ($P < 0.001$). Methane yield significantly decreased by 38.06% and 44.78% in steers fed diets containing AS4 and AS7, respectively ($P < 0.001$). Additionally, methane intensity was significantly reduced by 36.76% in steers fed AS4 and by 44.85% in those fed AS7 ($P < 0.01$). Overall, the findings of this study demonstrated that a 0.5% level diet inclusion of *Asparagopsis*-based feed additive with bromoform content of either 4 mg/g or 7 mg/g effectively reduced methane emissions from Hanwoo steers on their typical high concentrate diet without adversely impacting the rumen fermentation or growth performance.

177 Methane production, yield, and intensity of different diets with perennial ryegrass and tall fescue

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To safeguard the functionality of grasslands and ensure sustainable forage production, diversifying European grassland ecosystems becomes necessary. One promising species is tall fescue (*Festuca arundinacea* Schreb.). However, acknowledging certain limitations such as its relatively low digestibility and the limited voluntary intake by cows is crucial. The digestibility of the organic matter in perennial ryegrass (*Lolium perenne* L.) is approximately 8 percentage points higher than that of tall fescue harvested with the same frequency. This lower digestibility of tall fescue together with the lower nutritional value and higher NDF content can be a driver for higher methane production. This study aimed to evaluate the effects of tall fescue and the inclusion of red clover (*Trifolium pratense*) on methane production, yield, and intensity in dairy cows. We conducted four experiments using a GreenFeed system to measure methane emissions from dairy cows fed diets containing a high amount of grass silage, focusing on perennial ryegrass (diploid: **Lp2**; tetraploid: **Lp4**) and tall fescue (**Fa**), with or without red clover (**Tp**). In experiment 1, cows were fed Lp2, Lp4, and Fa. The enteric methane production (**CH₄**, g/day) did not differ between treatments, averaging 518, 511, and 517 g/day, respectively. However, methane yield (**CH₄**/kg dry matter intake, **DMI**) was significantly higher for Fa (23.7 g/kg) compared to Lp2 and Lp4 (22.8 and 22.4 g/kg, respectively). Likewise, methane intensity (**CH₄**/kg fat and protein corrected milk, **FPCM**) was significantly higher with Fa (15.8 g/kg) compared to Lp4 (14.6 g/kg). Experiment 2 included Lp2, Lp2+Tp, and Fa+Tp. Methane production was not different between diets, ranging from 519 (Lp2+Tp) to 534 g/day (Fa+Tp). Methane yield was lowest for Lp2+Tp (22.1 g/kg DMI) and significantly different from Lp2 (23.0 g/kg DMI), while Fa+Tp (22.3 g/kg DMI) was not significantly different from Lp2 nor Lp2+Tp. Methane intensity was not affected, remaining between 15.0 (Lp2+Tp) and 15.5 (Fa+Tp) g/kg FPCM. In Experiment 3, Lp2 was compared to Fa. Methane production was not different for Lp2 (480 g/day) compared to Fa (487 g/day). Methane yield was significantly reduced for Lp2 (18.4 g/kg DMI) compared to Fa (20.0 g/kg DMI). Similarly, methane intensity was lower for Lp2 (12.8 g/kg FPCM) than Fa (13.7 g/kg FPCM). Experiment 4 compared diets including Tp (Lp2+Tp and Fa+Tp). Methane production was significantly lower for Lp2+Tp (506 g/day) than Fa+Tp (519 g/day). Methane yield was also significantly reduced for Lp2+Tp (19.4 g/kg DMI) compared to Fa+Tp (20.4 g/kg DMI). Similarly, methane intensity in Lp2+Tp (12.6 g/kg FPCM) was significantly lower than Fa+Tp (13.6 g/kg FPCM). In conclusion, tall fescue exhibited higher methane emissions and especially higher methane yield and intensity due to a lower feed intake and lower milk production. However, these results also demonstrate that red clover inclusion in perennial ryegrass-based diets can effectively reduce methane yield and intensity, providing potential methane mitigation strategies.

182 Calcium peroxide effect on production, methane emissions and microbiota in dairy cows

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Oxidizing agents like calcium peroxide (**CaPe**) have been demonstrated to significantly reduce methanogenesis. We evaluated the effect of CaPe on enteric methane (**CH₄**) emissions, animal performance, rumen fermentation and rumen microbiota composition in lactating Nordic Red dairy cows. Four cows were used in a 4×4 Latin square experiment with 28-day experimental periods (21-day adaptation and 7-day sampling). Control diet (**CON**) comprised grass silage and concentrates (ratio 65:35) fed four times a day as total mixed ration (**TMR**). CaPe was supplemented to CON at 0.75 or 1.5% DM to form CaPe1 and CaPe2, respectively. Daily feed intake, milk yield and composition were recorded during the sampling period. Methane production was measured over 4 days in respiration chambers. Total faeces was collected to determine nutrient digestibility. Samples of rumen liquid were collected for volatile fatty acid (**VFA**) and rumen microbiota analysis. Sample chemical analyses and microbiota analyses were performed following standard protocols. Statistical analyses were performed by PROC GLIMMIX of SAS 9.4. using period and treatment as fixed effects and cow as random effect in the model. Dry matter (**DM**) and organic matter (**OM**) intakes were reduced numerically by CaPe1 and significantly ($P<0.05$) by CaPe2. Milk and energy corrected milk (**ECM**) yields were lower ($P<0.05$) for CaPe2 compared with the CON while milk composition was not affected ($P\geq 0.13$). Milk production efficiency expressed as milk energy divided by metabolizable energy intake was not affected ($P=0.50$) by the treatments. Apparent total tract digestibility of DM was lower ($P<0.01$) for CaPe treatments compared with CON. Digestibilities of OM, crude protein and ether extract were lower ($P\leq 0.03$) only for CaPe2 compared with CON. Digestibility of neutral detergent fiber was lower ($P<0.05$) for both CaPe1 and CaPe2 compared with CON. Apparent bioavailability of Ca and P was not affected ($P\geq 0.22$). Rumen pH and total VFA concentrations were not affected ($P\geq 0.27$) by treatment. Ruminal molar proportion of acetate was not affected while propionate tended ($P=0.06$) to be lower for CaPe2 than CaPe1. Daily CH₄ production was 12.4 and 14.3% lower ($P=0.02$) for CaPe1 and CaPe2 diets compared with CON. However, CH₄ yield (g/kg OM intake) or intensity (g/kg milk or ECM) were not different ($P\geq 0.14$) among treatments. Hydrogen production and yield were lower ($P<0.01$) for CaPe diets compared with CON. Rumen bacteria and ciliate protozoa were significantly ($P<0.05$) affected by CaPe2 treatment but not by CaPe1. In comparison to CON, CaPe2 reduced relative abundances of *Olsenella*, *Prevotella*, *Prevotellaceae* YAB2003 group, *Gastranaerophilales*, *Clostridia* UCG-014, *Butyrivibrio*, *Succinivibrionaceae*_UCG-002, and *Succiniclasticum*, but increased *Bacteroidales* p-251-o5, *Anaeroplasma*, *Erysipelatoclostridiaceae* UCG-004, *Solobacterium*, RF39, *Coproccoccus*, *Marvinbryantia*, and *Moryella*. In comparison to CON, CaPe2 increased relative abundances of *Entodinium* sp., and *Entodinium longinucleatum*, and decreased *Epidinium* sp., and *Isotricha* sp. Rumen archaea and anaerobic fungi were not affected by the treatments. Feeding CaPe supplemented diets as TMR four times daily had negative effects on DM intake and animal production traits. Feeding CaPe once a day should be tested to explore the full potential of CaPe as CH₄ inhibitor.

184 Impact of lactic acid bacteria inoculants on silage quality, microbial dynamics, and methane emissions mitigation

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Silage quality is essential for efficient ruminal fermentation and sustainable livestock systems, but challenges such as nutrient loss and environmental impacts must be addressed. This study evaluated the effect of three lactic acid bacteria (LAB) inoculants – **Ecosyl** [*Lactiplantibacillus plantarum* MTD/1 (NCIMB 40027)], **PJB1** [*Lentilactobacillus buchneri* PJB/1 (NCIMB 30139)], and **Ecocool** (mix of both) – on the quality, microbiome dynamics, and ensiling fermentation characteristics of perennial ryegrass (PRG)-based silages. The PRG was harvested, sieved (5–7 cm) and sprayed with LAB suspension (Ecosyl $\sim 1 \times 10^6$ CFU/g; Ecocool $\sim 3 \times 10^5$ CFU/g and PJB1 $\sim 2 \times 10^5$ CFU/g) or sterile water as control (CON). Samples were stored in airtight jars at room temperature and analysed at 0, 4, 24, 48h; 7, 14, 28, 90d for pH, volatile fatty acids (VFAs), fresh weight change and microbial diversity. The impact of inoculants on rumen fermentation and methane emissions was evaluated through *in vitro* incubation of 90-day silage samples in 100mL serum bottles with pooled rumen fluid (1:1; four animals) and Van Soest buffer at 39°C, measuring gas pressure, pH, methane production via gas chromatography (20mL/sample), and ammonia concentration. Active bacterial communities were analysed using 16S rRNA gene PacBio CCS full-length sequencing, processed with QIIME2, excluding ≤ 10 counts samples/species. Taxonomic identities were assigned to ASVs using GreenGenes2. Alpha diversity was evaluated via Chao1, Shannon, and Simpson indices. Beta diversity was assessed with Bray-Curtis dissimilarity, visualized through PCoA, and tested for significance using PERMANOVA and Wilcoxon rank-sum. In the silage, Ecosyl and Ecocool produced the highest lactic acid concentrations ($P < 0.01$), significantly increasing its percentage in fermentation products relative to total VFAs, while retaining more DM than PJB1 and CON ($P < 0.05$). Ecosyl and Ecocool maintained significantly lower pH than PJB1 and CON throughout the study ($P < 0.05$). Weight significantly changed at 7, 28, and 90d ($P < 0.05$). Ecosyl and Ecocool produced the highest VFAs levels, particularly at 90d ($P < 0.01$), with acetic acid most abundant. Ethanol production was significantly higher in PJB1 at 90d ($P < 0.05$), while CON showed lower levels VFAs and ethanol. Ecosyl and PJB1 showed distinct microbial profiles than CON and Ecocool. The PCoA plots showed significant clustering by treatment $\times$ time ($P < 0.05$), indicating that both factors significantly influenced bacterial composition. Alpha diversity revealed Ecosyl and PJB1 enhanced bacterial richness (Chao1) and diversity (Shannon, Simpson) than CON ($P < 0.05$). Microbial diversity increased early (14d) and stabilised or decreased later (28–90d). In the rumen, PJB1 reduced gas pressure ($P < 0.05$). Ammonia increased uniformly over time ($P > 0.05$). Ecocool and Ecosyl maintained significantly lower pH than PJB1 and CON throughout 24 and 48h ($P < 0.05$). PJB1 significantly reduced methane than CON at 4h (51.76%; $P < 0.05$), 24h (64.96%; $P < 0.01$), and 48h (51.62%; $P < 0.01$), showing the greatest reduction over time. Ecocool also reduced methane levels but to a lesser extent 4h (33.82%; $P < 0.05$), 24h (23.39%; $P < 0.01$), and 48h (48.47%; $P < 0.01$), indicating their potential to reduce GHG emissions. Overall, the findings suggest that the LAB inoculants evaluated can enhance silage fermentation, nutrient retention, microbial stability, and potentially reduce methane emissions, supporting sustainable livestock systems.

188 3-Nitrooxypropanol reduced enteric methane of dairy cows more when fed in mixed than separate diets

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A 4×4 Latin square experiment was conducted to examine the efficacy of 3-NOP fed separately in the concentrate (**SEP**) or in total mixed ration (**TMR**) on enteric methane production, its diurnal variation, and rumen microbiome. The dietary treatments were arranged as a 2×2 factorial arrangement i.e., SEP vs. TMR feeding (grass silage and concentrate 65:35 on dry matter, **DM**, basis), and 0 vs. 70 mg 3-NOP/kg diet DM. Four multiparous Nordic Red dairy cows were used in respiration chambers including four 21-d periods (17 d adaptation and 4 d sampling). Cows were fed at 07:00, 13:00, 17:00 and 19:00. 3-NOP was mixed directly into concentrates twice daily (07:00 and 19:00) for SEP and to the mixer for TMR. Feed intake, milk production and milk composition were measured during sampling period. Rumen samples were taken at 10 AM in the last day of each period using a stomach tube for rumen VFA and microbiota analysis. Results were analysed with Proc GLIMMIX of SAS 9.4. using period, 3-NOP, feeding method and 3-NOP × feeding method interaction as fixed effects and cow as a random effect in the model. Dry matter intake was reduced by 3-NOP (21.2 vs. 22.4 kg/d; $P<0.05$) and the cows fed SEP diets had lower DM intake compared with those fed TMR (21.0 vs. 22.5 kg/d; $P<0.05$). Feeding method or 3-NOP did not affect milk and energy corrected milk (**ECM**) yields, milk composition, and feed efficiency calculated as milk or ECM yield divided by DM intake. 3-NOP reduced methane production (g/d) by 24.4% and 10.7%, methane yield (g/kg DM intake) by 16.9% and 9.4% and methane intensity (g/kg ECM) by 21.4% and 9.0%, when used in TMR or SEP diets, respectively ($P<0.01$ for the interaction). 3-NOP decreased ($P<0.01$) ruminal molar proportion of acetate, and increased propionate, butyrate, and isobutyrate. There was a noticeable drop in methane production when cows were fed SEP diet with NOP at 07:00 and 19:00 followed by a peak shortly after depletion of 3-NOP in the rumen. When cows were fed TMR with NOP, the methane production stayed consistently lower compared to TMR without NOP throughout the day. 3-NOP increased ($P<0.05$) relative abundance of *Bacteroidota* sp., *Muribaculaceae* sp., *Lachnospiraceae* NK3A20 group, *Oscillospiraceae* sp., *Anaerovorax*, and *Mogibacterium* but decreased abundance of *Lachnospiraceae* NK4A136 group, [*Eubacterium*] *xylanophilum* group, [*Ruminococcus*] *gauvreauui* group, *Oscillospiraceae* UCG-005, *Ruminococcaceae* CAG-352 and *Polyplastron*. No significant effect was observed on rumen archaea or anaerobic fungi. In conclusion, 3-NOP was effective in reducing enteric methane emissions but the lower methane reduction with the TMR diet compared with international studies (21.4 vs. 30% reduction in methane intensity) may be due to the relatively high NDF content of the grass silage-based diet used in this study. Only 42% of the full potential of 3-NOP in methane intensity reduction can be achieved if 3-NOP is fed twice a day in separately fed diets compared to TMR feeding.

189 Low nitrogen feeding reduce environmental impacts of pig production from the feeding to the effluent

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Livestock production is associated with a number of environmental impacts, such as climate change, acidification and eutrophication. These impacts are particularly marked in areas with high livestock densities. Gas emissions from effluents are implicated in acidification and eutrophication in the case of ammonia (**NH₃**), and in climate change in the case of methane (**CH₄**) and nitrous oxide (**N₂O**). Emissions of NH₃ and N₂O from effluent are linked to the excretion of nitrogen (**N**) in urine and faeces. While previous studies have shown that reducing dietary protein content is an efficient way to reduce N excretion and associated NH₃ emissions. Moreover, decreasing dietary crude protein (**CP**) contents in pig diet is a way to decrease the use of imported soybean meal which is associated with deforestation and high climate change impact. The objective of this study was to investigate the effect of a diet with low protein content in reducing environmental impacts of pig production at the entire feed-animal-effluent system. Diets with different contents of CP: 15% (Control-diet with 10% of soybean meal) vs 12% (Low-CP-diet with no soybean meal), were formulated. A total of 12 Pietrain x (Large White x Landrace) non-castrated pigs (mean body weight: 40±5 kg) were used in this study and received one of the two experimental diets (n=6 per experimental diet). Digestibility cages and respiration chambers were used to measure nutrient intake, energy and nitrogen utilisation, and NH₃ emissions. We also measured NH₃ and CH₄ emissions on collected slurry. Experimental datas were subjected to an analysis of variance that tested the effects of diet (R software). Results were considered significant for P-values lower than 0.05. Our pig feeding strategy of N reduction decreased climate change impact by 17%, non-renewable energy use by 4%, eutrophication by 14% and land occupation by 5% per kg of feed. The low-CP-diet decreased the N excreted in urine per pig per day by 25%. We also observed lower total N content (-19%), total ammonia nitrogen content (-33%) and pH-value of slurry (-0.22) with the reduction of CP in diet with no modification in the amount of fresh faeces and urine excreted per pig per day. By consequences, in our experimental housing and storage conditions, the Low-CP-diet reduced NH₃ emission from slurry by 30% with no modification in CH₄ production. Nutrient digestibility (-2.3%) and total heat production (-5%) were lower with the low-CP-diet which resulted in lower protein deposition (-25%) and higher fat deposition (+16%). Our feeding strategy of N reduction is efficient to reduce environmental impacts of pig diet and NH₃ emissions from slurry. However, we need to perform a life cycle assessment study which integrates the long-term data of animal performance, excretion, emissions and environmental impacts in order to find the best compromise between economic and environmental goals of pig production.

191 GLEA-Stat Feed: A new dataset of greenhouse gas emission factors for animal feed commodities

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A detailed accounting of feed-related greenhouse gas (GHG) impacts is necessary to adequately assess the environmental performance of livestock supply chains. This both ensures accurate estimates of baseline emissions and helps identify entry points for mitigation interventions. Despite the relevance of emissions related to feed, the existing data sources are rather scattered and either do not include all agricultural commodities and byproducts, or do not provide comprehensive information on their origin. The latter aspect is of particular importance, as it determines the underlying production systems and GHG emissions in a context of international trade dynamics. Representing the diversity of feed commodities used across animal production systems and countries is crucial for a consistent, systematic, and comprehensive analysis. In this assessment, we estimate GHG emission factors associated with feed production, expressed as kg of CO₂-equivalents per kg of feed commodity. GHG emissions are estimated under a life cycle assessment approach, including life cycle emissions from cradle to animal mouth and using 2020 as a reference year. To do so, the latest available global datasets and the IPCC guidelines are used to estimate GHG emissions related to crop production, for the following emission sources: 1) CO₂ emissions from the energy used for on-farm agricultural activities and for the manufacture of inputs, such as synthetic fertilizers and pesticides; 2) N₂O emissions associated with nitrogen inputs to soil, such as synthetic fertilizers, manure and crop residues; 3) CH₄ emissions from rice cultivation; and 5) CO₂ emissions from deforestation and peat drainage due to croplands expansion. Emissions from tillage activities of different soil types were not included at the current stage, because of the lack of consistent and comprehensive data sources at global scale. This information is then combined with a state-of-the-art database on bilateral trade and footprints of feed commodities. The resulting emission factors are provided for more than 200 crop-based feed commodities used globally, by country of consumption and taking into account the diversity of countries of origin and supply chains. The methodology and data presented constitute the first open-access resource providing global coverage of emission factors for feed items. A consistent methodology is used across all countries, in alignment with official statistics from the Food and Agriculture Organization of the United Nations (FAO) and facilitating future updates. Future areas of work will include the evaluation of the uncertainty of results, the inclusion of additional feed commodities and the expansion of the emission sources and environmental impacts considered.

192 Sustainable Milk Production with Apple and Hempseed by-products: Mitigating Emissions from Rumen to Manure in Dairy Farming

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Ruminant animals can utilise human-inedible foods to supply essential nutrients to human diets but inevitably produce considerable emissions of nitrogen (N) and methane (CH₄) throughout the production chain. Incorporating agro-industrial by-products into ruminant feed is an alternative owing to cost effectiveness, adding nutritional value and mitigating emissions. Apple pomace and hempseed, rich in bioactive compounds like polyphenols and tannins, have great potential for reducing ammonia and CH₄ emissions. The aim of this study was to explore effects of feeding dairy cows apple pomace and hempseed on enteric and manure gas emissions. Apple pomace was collected and ensiled with 30% wheat straw (DM basis) for three months. Hempseed cakes were acquired from Hemp Co., Ltd. Fifteen mid-lactation Holstein dairy cows were used in a 3 (diet) x 3 (period) Latin Square design study and each period lasted 24 days including diets adaptation and data collection. Three diets comprised 10% inclusion of different by-products in total mixed ration (TMR): (1) Control diet, basal TMR including 45% grass silage, 5% wheat straw and 50% concentrates; (2) Apple pomace diet, 10% grass silage replaced by apple pomace in TMR; (3) Hempseed cake diet, 10% concentrates replaced by hempseed cake in TMR. In each period cows were housed in a cubical accommodation as a single group for the first 17 days with CH₄ emissions measured using a Greenfeed unit, and then kept in digestibility units, where total faeces and urine outputs were collected. Faeces and urine samples from each cow were completely mixed and then stored in a temperature-controlled chamber at 12 °C for gas emissions measurements at a series of timepoints within 4 weeks. Data were analysed using the mixed model procedure of SPSS (Version 22.0, IBM), with post-hoc Fisher's Least Significant Difference test ($P < 0.05$). Feeding apple pomace and hempseed cake significantly decreased enteric CH₄ production by 6.3% and 6.7% respectively ($P < 0.05$). For intensities, CH₄/DM intake were reduced by 10.6% and 10.1% with dietary inclusion of apple pomace and hempseed cake, while CH₄/milk yield were reduced by 9.8% and 10.9% ($P < 0.01$). Inclusion of apple pomace decreased urine and manure N excretion/milk yield by 13.8% and 8.2% ($P < 0.05$), while similar reduction (16.3 % and 10.9%, $P < 0.05$) was also observed in hempseed cake group. For the manure storage experiment, apple pomace diet significantly reduced NH₃ and CH₄ fluxes at a series of timepoints, thus reduced cumulative NH₃ and CH₄ emissions over 4 weeks manure storage by 24.8% and 27.4% respectively, compared to the control diet ($P < 0.05$). However, feeding hempseed cake had no significant effects on manure NH₃ and CH₄ emissions when compared to control and apple pomace diets. Dietary inclusion of apple pomace and hempseed cake could be a potential strategy to mitigate enteric methane emissions from dairy cows, while apple pomace could further contribute to less ammonia and methane emissions from manure storage.

195 Nutrient composition of tropical pastures in Tailevu province, Fiji

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Dairy cattle are major source of methane emissions. Improving pasture quality can improve animal production efficiency and reduce methane emissions. This study evaluated the nutritive value of three grass species available in Fiji, for use in dairy production. Setaria (*Setaria sphacelate*), Para grass (*Brachiaria mutica*), and Juncao grass (*Pennisetum* hybrid). There were four samples of each grass species collected in October and November in Tailevu province. Dry matter (**DM**) content of pasture was determined by drying at 105 °C for 72 hours. The chemical composition, fibre fraction, mineral profiles and metabolisable energy were analysed after drying at 65 °C for 72 hours at the commercial lab using standard procedures.

The characteristics of Setaria, Para grass and Juncao grass were tested respectively, including DM (164 g kg⁻¹, 214 g kg⁻¹, 190 g kg⁻¹), crude protein (67 g kg⁻¹, 72 g kg⁻¹, 71 g kg⁻¹), neutral detergent fibre digestibility (679 g kg⁻¹, 657 g kg⁻¹, 688 g kg⁻¹), acid detergent fibre (417 g kg⁻¹, 446 g kg⁻¹, 422 g kg⁻¹), lignin (72 g kg⁻¹, 92 g kg⁻¹, 62 g kg⁻¹), total digestible nutrient (513g kg⁻¹, 490 g kg⁻¹, 510 g kg⁻¹), and metabolisable energy content (7.5 MJ kg⁻¹, 7.1 MJ kg⁻¹, 7.5 MJ kg⁻¹). Para grass had significantly higher lignin content than Setaria and Juncao grass ($P < 0.01$). The range of minerals were as follows for Setaria, Para grass and Juncao, respectively: Ca 2.7 g kg⁻¹ - 4.4 g kg⁻¹, Mg 2.5 g kg⁻¹ - 4.2 g kg⁻¹, K 11 g kg⁻¹ - 25 g kg⁻¹, Na 0.6 g kg⁻¹ - 7.1g kg⁻¹, Fe 342 mg kg⁻¹ - 1193 mg kg⁻¹, Zn 40 - 49 mg kg⁻¹, and Cu 7.8 mg kg⁻¹ - 13.5 mg kg⁻¹. Setaria had significantly higher Mg ($P < 0.01$), Na ($P < 0.01$), and S ($P < 0.05$), content than Para grass and Juncao grass. Para grass had significantly higher Cu ($P < 0.01$) content than Setaria and Juncao grass, higher Ca ($P < 0.05$) than Setaria, and higher Fe ($P < 0.05$) than Juncao grass. Juncao had significantly higher K ($P < 0.01$) content than Setaria and Para grass. Dairy cows in Fiji are grazed on poor quality pasture. Improving pasture digestibility can reduce methane yield from DM intake and improve methane intensity.

The three pastures were shown to have similar nutritive content and NDF, however, understanding and balancing minerals requirements can enhance animal health and production efficiency, and maintain long-term emission intensity outcomes

196 Feeding an essential oil mix reduces enteric methane emissions in grazing sheep

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Australia, being the world's largest sheep exporter, and with its sheep meat production volumes expected to increase by 3% by 2024-2025, strategies for reducing enteric methane emissions to support sustainable sheep production are required (Coulthard et al., 2024). This study aimed to assess whether a commercially available essential oil blend could reduce enteric methane emissions and improve body weight gain in sheep over a longer period and under grazing conditions, building on previous short-term experiment results (Prathap et al, 2024). Forty-eight Merino sheep, aged 2-3 years, were randomly allocated into two groups based on body weight: a control group (n=24) and a treatment group (n=24). The study was conducted over a period of 3 months at the University of Melbourne Dookie Campus Sheep Farm, Victoria Australia. The use of animals for this research and all the protocols were approved by the University of Melbourne Animal Ethics Committee (**AEC** ID: 28286). Sheep were adapted to the Greenfeed emission monitoring system and pellet feeding over 3 weeks period. Sheep were grazing perennial rye grass pasture and supplemented with mixed forage hay and lamb pellets. The treatment group received standard lamb pellets incorporated with an essential oil mix (Agolin® ruminant; dosage 0.2 g/day) while the control group received standard lamb pellets. The GreenFeed Emission Monitoring (**GEM**) system (C-Lock Pvt Ltd) was used to measure GHG emissions from the experimental sheep over a 9-week period, employing a 2-day rotational cycle, where each group spent two consecutive days in a paddock with the GEM system before being replaced by the next group. In total, each group underwent 12 effective 2-day cycles. The treatment pellets were used to entice the sheep to visit the GEM system multiple times daily. During a visit to the GEM Unit station, sheep are encouraged to enter an enclosed area or individual feeding stall where enteric CH₄, CO₂, O₂, H₂, and H₂S measurements are taken. Sheep body weight was recorded at fortnightly intervals, and average daily gain was calculated. All statistical analyses were performed using GenStat 16th edition (Version 16.1.0.10916, VSN International Ltd, United Kingdom). The REML analysis procedure was used to test for significant differences among the groups, with groups considered as the fixed model effect and animal number as random variable. A p-value of ≤ 0.05 was considered significant. The treatment group (47.9 g/day) showed a 25% reduction (p = 0.029; SED: 6.35) in daily methane production compared to the controls (63.5 g/day). There was a trend (p = 0.12; SED: 9.15) for increase in average daily gain (g/day) following Agolin supplementation and the lambs in treatment group showed higher average daily gain (105.81 g/day) as compared to control group (91.62 g/day). Based on these results it can be concluded that essential oil mix could be used as a potential feed additive to reduce enteric methane emissions in sheep. Future studies should incorporate sward structural variables and pasture intake to further investigate the impact of feed additives on CH₄ emissions.

197 Identifying a stable carrier for bromoform to deliver enteric methane mitigation

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The halogenated compound bromoform has demonstrated consistent methane mitigation for ruminant livestock fed total-mixed rations. However, bromoform is inherently volatile making delivery challenging. A stabilising carrier is required to facilitate mitigation outcomes on farm and incorporation into supplementary feeds. The objective of this study was to assess the extent of methane mitigation in vitro of three inert carriers loaded with bromoform and select the most suitable to progress to in vivo studies.

The artificial rumen simulation technique (RUSITEC) was used to assess the methane mitigation from three activated carbons. Carrier B was chosen based on its effectiveness in a previous RUSITEC. Carriers A and C had similar bromoform desorption kinetics to carrier B in a benchtop test. Each carrier was loaded with 10% bromoform (w/w) and dosed daily in vitro at two levels (low, 1.5 μM ; **L** and high, 2.5 μM ; **H**), creating six treatments; **AL**, **AH**, **BL**, **BH**, **CL**, **CH** equivalent to 6.3 and 12.6 mg CHBr_3 /kg DM for low and high doses, respectively. The 2.5 μM dose previously demonstrated complete inhibition of methane in a RUSITEC with carrier B, and 1.5 μM was included to elucidate differences between carriers. A substrate only treatment (30 g oaten chaff) was used as a control. Total gas produced in each fermentor was measured daily and sub-sampled for methane quantification over two, 14 d periods. Data were analysed as a nonlinear mixed effects design using the nlme package in R. Individual fermentor and run were considered random effects with treatment a fixed effect. Pairwise comparisons between treatments were performed using the emmeans package with a Tukey adjustment for multiple comparisons.

All treatments significantly reduced methane yield (**MY**) compared with the control ($P < 0.05$) over the 14 d incubation. Treatments BL, BH and CH had the greatest effect, inhibiting more than 99% of MY compared with the control after 14 d. The modelled rate of MY mitigation per 24 h of these treatments were 40%, 50% and 44% ($P < 0.05$), respectively. Treatment AH also demonstrated significant mitigation ($> 97\%$ of MY) particularly from day 7-14. The carrier B demonstrated similar mitigation effect ($P > 0.05$) regardless of high or low CHBr_3 concentration. Treatments AL and CL demonstrated the smallest mitigation effect, with a rate of decrease in MY of 3% and 6% per 24 h period, respectively. There was a significant difference in MY between the high and low concentrations of carriers A and C.

The mitigation demonstrated by the three carriers in the RUSITEC is predicated on activated carbon reducing bromoform's volatility while allowing for its slow release in the rumen environment. The RUSITEC does not directly translate to outcomes in vivo, but has enabled a comparison of the characteristics of the carriers and confidence in an extended effect over 14 d. Carrier B was the most effective carrier, with significant mitigation across concentrations indicating an ability to retain bromoform up to 2.5 μM or deliver a daily dose of 12.6 mg CHBr_3 /kg DM.

200 Effects of dietary protein levels on ammonia and nitrous oxide emissions from dairy cows manure

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Livestock slurry storage contributes considerably to global emissions of ammonia (NH_3) and nitrous oxide (N_2O). Dietary manipulation of crude protein (**CP**) levels is considered an effective strategy to reduce nitrogen (**N**) excretion and NH_3 and N_2O emissions from dairy systems. The objective of this study was to examine the impact of dietary CP levels in dairy cow diets on manure N excretion and on subsequent NH_3 and N_2O emissions from the manure. Three manure incubation experiments were conducted at the Agri-Food and Biosciences Institute, Northern Ireland. Manure samples were collected from three feeding experiments with lactating Holstein dairy cows. In each experiment cows were offered diets (**TMR**) containing various CP levels (14.0 - 17.5% (DM basis)) which were achieved by manipulation of concentrate CP concentrations. During each experiment 9 - 12 cows were used in digestibility measurements with total faeces and urine outputs collected. Urine output from each cow was collected in a sealed container (no acid added) surrounded by ice to minimise N loss. Faeces and urine from each cow were then mixed in the same proportion (by weight) as they were excreted (total of 15 kg) in a 30 L bucket and a manure sample was taken at the start of incubation. Buckets were stored in a controlled temperature chamber at 14 °C with bucket lids placed to cover approximately $\frac{3}{4}$ of the opening to prevent the sample drying excessively. NH_3 and N_2O emissions were measured at 0, 3, 6, 24, 48, 72 and 96 hours following mixing, and subsequently at weeks 1, 2, 3 and 4 using the static chamber technique. During each measurement period each bucket was sealed using a lid for one hour before the measurement of NH_3 and N_2O concentrations using a photoacoustic gas analyser connected to the bucket headspace with PTFE tubes. Gas emission rates were calculated and reported as $\text{g m}^{-2} \text{h}^{-1}$. The data were analysed using REML (GenStat, 21st ed., VSNi Ltd) with overall mean, cow, sampling time, treatment, and error term included in the model. Diets was a fixed effect, whereas all other factors were random effects. Reducing dietary CP levels decreased N content in urine and N excretion in faeces and urine as a proportion of N intake ($P < 0.05$). Ammonia emission rate increased rapidly after manure mixing, peaking at 24 h for most diet treatments, before decreasing gradually until the end of the measurement period. The peak N_2O emission rate for all diets were observed at first measurement (0 h), and then reduced afterwards. Reducing dietary CP levels reduced NH_3 emission rates by an average of 43% (range from 51% to 14%). However, lowering dietary CP levels didn't affect N_2O emission rates in this incubation study. Reducing dietary CP levels is an effective strategy to mitigate NH_3 emissions from dairy manure but had no impact on manure N_2O emissions.

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201 Validating the use of an inert carrier for bromoform to deliver mitigation outcomes in cattle

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Strategies aimed at reducing methanogenesis have the most utility to achieve abatement targets for livestock. Candidate inhibitors can be structural analogues of methyl-donor compounds (namely CHBr_3) targeting the methyltransferase complex or analogues of methyl-coenzyme M, inhibiting methyl-coenzyme M reductase enzyme. While bromoform has demonstrated consistent methane mitigation for ruminant livestock fed total-mixed rations or high-grain diets, a stable inert carrier is required to facilitate consistent mitigation outcomes on farm and into existing supplementation practices. This study assessed the efficacy and persistence of mitigation from bromoform presented in an inert solid carrier when fed to beef cattle consuming a forage diet. Sixteen *Bos indicus* heifers (mean LW $\pm$ SD: 357 $\pm$ 18.4 kg) fed Rhodes grass hay ad lib for 14 d were allocated to four treatment groups of control (0), low (62), medium (128), high (194) bromoform dose rates (mg/d). Sub samples of the diet were pooled for proximate analysis. A solid inert carrier was used to deliver the bromoform (10% w/w) each morning for a subsequent 10 d before individual animal methane production was measured over 48 h using open circuit respiration chambers. Methane production (**MP**, g/d) corrected to STP was converted to daily methane yield (**MY**, g/kg DM intake) based on individual animal dry matter intake (**DMI**). Data were analysed for difference in MP and MY using ANOVA in R and accounting for treatment, groups and chamber as fixed effect.

The inclusion of an inert carrier containing 10% w/w bromoform had no significant effect ($P > 0.05$) on DM intake which ranged from 5.1 to 5.8 kg/d (mean $\pm$ sem: 5.4 $\pm$ 0.11 kg/d). Methane production and MY from animals receiving the high dose of CHBr_3 was significantly different to control and all other levels of inclusion ($P < 0.001$). A 22% reduction was observed for methane yield at this level (17.8 $\pm$ 0.46 g/kg DMI) compared with control (22.8 $\pm$ 0.31 g/kg DMI). One noticeable feature of this formulation was the absolute extent of mitigation over time post feeding compared with an alternative soluble formulation. The effectiveness of the inert carrier formulation was double with approximately 10 h of inhibition observed for animals in chambers compared with a soluble formulation.

The mitigation achieved in this study was not associated with a decrease in feed intake, suggesting that animal productivity would not be impacted. Animals in the high dose group exhibited the greatest extent of mitigation from once a day feeding. This was associated with a dietary inclusion of 36 mg/kg DMI compared with the medium (25 mg/kg DMI) or low (12 mg/kg DMI) inclusion rates. Notable was the extended period of mitigation achieved which was greater than the short term (< 3 h) effect typically associated with other antimethanogenic compounds. Inclusion of a granulated carrier would be feasible on farm in existing supplementation practices. This outcome suggests an ability for the carrier to retain bromoform within its matrix compared with complete release soon after feeding resulting in greater, but short term mitigation.

202 Appropriate strategies that reduce emission intensity of livestock enterprises can improve resilience and profit

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Farmers are under increasing pressure to address climate challenges such as increasing climate variability and greenhouse gases. There is little that extensive livestock farmers can do currently to reduce total emissions without reducing stock numbers and productivity. Emission intensity (**EI**; emission per kg of product produced) is of increasing interest as a measure of farm efficiency and by supply chains wanting to produce low emission products.

Four south-eastern Australian case study farms, representing a range in land class and rainfall, were developed in the GrassGro™ model to reflect actual pasture growth, land type and variability. 14 enterprises (sheep and beef) plus a number of common strategic management options used to improve performance of the different enterprises, were run over a 40 year time period to assess measures of profitability; productivity; methane emissions and flexibility. Management strategies that reduced the effects of the poor years (higher profit/low grass production) and increased or did not reduce profits in the good years, were considered to have greater flexibility.

Known strategies such as increasing fecundity, improving the feedbase and/or genotypes reduced EI at all sites, but they were not the same across sites. Improving sheep weaning rates changed EI between +2% to -16%. Improved pastures increased total emissions but had one of the highest impacts on reducing EI for prime lamb production (-22 to -27%). Feeding grain to finish lambs in terminal systems, only reduced EI as pasture quality decreased. Differences were less at more variable and/or poorer land class environments.

Wool enterprises, as less sensitive to nutrition, provided a buffer to the susceptibility to droughts and fared better in EI and profit at the lower rainfall and poorer land class sites. Incorporating some meat production by joining a proportion of ewes to maternal or terminal sires, improved EI by -3 to -11%, while adding flexibility to the production system. However, current measures of EI for wool do not incorporate micron, as the main determinant of price and wool quality; an area for future research.

Strategies that reduced EI were also most profitable when flexibility rather than profit maximisation was prioritised. Therefore, the most efficient systems, in terms of EI, that were also highly profitable tended to be those that maximised returns in good years and reduced susceptibility to low rainfall years, compared to those that focussed on profitability alone.

If farmers make enterprise and management strategies on profitability appropriate to the feed base, but also with the flexibility to deal with seasonal extremes in pasture production, then these choices will also inherently lead to lower average EI. Understanding these relationships will be important as supply chains increasingly demand lower emissions per product. Further research is required to provide metrics on EI for shared products (e.g. meat and wool) that reflect the efficiencies of each.

203 Effect of oaten hay quality in TMR diets on in-vitro methane production and rumen fermentation

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Feed quality optimization for methane mitigation presents a significant challenge in ruminant production systems. This study investigated how water-soluble carbohydrates (**WSC**), neutral detergent fibre digestibility (**NDFD**) and neutral detergent fibre (**NDF**) of oaten hay influence *in vitro* rumen fermentation dynamics and methane production in total mixed ration (**TMR**).

The experiment followed a randomized complete block design with three treatments and eight replications per treatment including three blanks across two experimental runs. The Blank modules containing rumen fluid without substrate were included to account for baseline gas and methane production. Using a 48-hour *in vitro* approach with the Ankom Gas Production system (ANKOM Corp., Fairport, NY, USA), we evaluated TMR formulations containing 15% oaten hay of varying quality (High: WSC 25.9%, NDFD 64.0%, NDF 50.68%; Medium: WSC 16.7%, NDFD 55.0%, NDF 57.91%; Low: WSC 12.7%, NDFD 54.0%, NDF 59.73%). All TMR diets maintained identical proportions of other ingredients (19.52% barley hay, 17.37% alfalfa hay, 15.38% faba bean, 25.04% wheat grain, and 7.69% molasses on a DM basis). The High, Medium, and Low-quality oaten hay diets had Neutral Detergent Fiber = 39.5, 41.9, & 42.5%; Crude Protein = 12.5, 12.7, & 13.3%; and Metabolizable Energy = 9.8, 9.7, & 9.6 MJ/kg DM, respectively. The statistical analyses were performed using GenStat 19th edition (VSN International Ltd., Hemel Hempstead, UK). Data were analyzed using one-way ANOVA with Fisher's LSD test for means comparison.

Results demonstrated that high-quality oaten hay significantly increased total volatile fatty acid production (51.8 ± 1.6 vs. 43.1 ± 1.6 mM; $P < 0.05$) and acetate: propionate ratio (2.70 ± 0.05 vs. 2.45 ± 0.05 ; $P < 0.05$) compared to low-quality hay. Methane production increased alongside improved fermentation parameters, with high-quality hay producing more methane per gram of dry matter than medium and low-quality hay (7.6 ± 0.4 vs. 5.8 ± 0.4 vs. 5.1 ± 0.4 mL/g DM; $P < 0.05$), as well as higher methane as percentage of total gas ($9.5 \pm 0.2\%$ vs. $8.1 \pm 0.2\%$ vs. $8.0 \pm 0.2\%$; $P < 0.05$). No significant differences were observed in total gas production, *in vitro* dry matter digestibility (0.55 ± 0.004 vs. 0.54 ± 0.004 vs. 0.53 ± 0.004 g/g DM; $P = 0.335$) or ammonia nitrogen concentrations (181.6 ± 3.4 vs. 178.6 ± 3.4 vs. 176.5 ± 3.4 mg/100mL; $P = 0.841$) across treatments.

These findings demonstrate that enhanced WSC, and NDFD; and reduced NDF characteristics in oaten hay simultaneously improved fermentation and increased methane production per unit of DM and gas production in the *in vitro* system. Further research is needed to determine how these observations translate to production environments and their implications for greenhouse gas mitigation strategies in ruminant production systems.

208 Methane prediction for different cattle categories fed fresh pasture

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Enteric methane emissions in the New Zealand greenhouse gas inventory are predicted using a fixed factor of 21.6 g methane/kg dry matter intake (**DMI**) (i.e., CH₄ yield) for all cattle categories (breed, age, physiological status). Generally, DMI is greater and pasture (predominantly perennial ryegrass) quality better for lactating dairy cows than in non-lactating cattle categories, which are factors that might affect CH₄ yield. The objective of the current study was to combine all available data from New Zealand studies on methane emissions from all cattle categories fed pasture as a sole diet, from measurements with respiration chambers, the SF₆ tracer technique and GreenFeed units, and develop CH₄ prediction equations. The final dataset contained data from 1921 individual cattle measurements across all seasons of the year for 99 unique study groups. 74% of the data was from lactating dairy cows, 8% from cattle between 0.25 and 1 year of age, 11% from cattle between 1 and 2 years of age and 7% from non-lactating cattle greater than 2 years of age. 15% of the data was from beef cattle (85% of growing cattle). All dairy cattle were female and 40% of beef cattle was female and 60% male (steers). Enteric methane prediction models (g/d and g/kg DMI) were generated with REML in GenStat version 23 with the study as a random effect in the model. The best models were selected based on Akaike information criterion (**AIC**) and residual mean squared prediction error (**RMSPE**) was calculated. The main findings of the analyses were that CH₄ yield decreased with increasing feeding level (DMI/body weight (**BW**)), that the relationship of CH₄ yield and DMI/BW differed between lactating and non-lactating cattle categories and that adding pasture chemical composition parameters (e.g. crude protein, fibre parameters, non-structural carbohydrate parameters, digestibility) to the statistical models did not improve methane predictions in cattle fed pasture only. Methane production (g/d) in lactating dairy cows could be predicted as $120.3 \text{ (standard error (SE) } 13.2) + 6.632 \text{ (SE } 0.59) \times \text{DMI (kg/d)} + 0.2033 \text{ (SE } 0.025) \times \text{BW (kg)}$ (AIC = 11985; RMSPE = 8.0%) and in non-lactating cattle as $7.505 \text{ (SE } 7.37) + 12.38 \text{ (SE } 0.69) \times \text{DMI (kg/d)} + 0.1769 \text{ (SE } 0.021) \times \text{BW (kg)}$ (AIC = 3330; RMSPE = 18.6%). Methane yield (g/kg DMI) could be predicted as $31.64 \text{ (SE } 0.61) - 3.266 \text{ (SE } 0.161) \times \text{DMI/BW}$ (AIC 4216; RMSPE = 16.5%) and in non-lactating cattle as $33.91 \text{ (SE } 0.81) - 5.303 \text{ (SE } 0.304) \times \text{DMIB/BW}$ (AIC = 1400; RMSPE = 17.1%) It is clear from the current analysis that the single fixed emission factor currently used in the national GHG inventory for cattle in New Zealand needs to be updated to improve national enteric methane accounting.

213 *In vitro* screening of the antimethanogenic effects of Patagonian seaweeds in ruminants.

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Enteric methane (**CH₄**) emissions are a significant contributor to global greenhouse gas emissions, particularly in livestock systems. In recent years, the use of marine macroalgae as a strategy to mitigate these emissions has gained notable attention within the scientific community. Over 200 species of macroalgae inhabit the Argentine coast, with the Patagonian region hosting the greatest diversity and abundance. To evaluate their potential to inhibit enteric methane emissions, an exploratory trial was conducted using 6 macroalgae species harvested along the Argentine coast.

The algae were freeze-dried, ground, and passed through a 2-mm screen. The species studied included *Undaria pinnatifida* - Sporophyll (**UPE**), *Undaria pinnatifida* - Blade (**UPL**), *Hymenena laciniata* (**HL**), *Phycodrys quercifolia* (**PQ**), *Dictyota dichotoma* (**DD**), *Pyropia columbina* (**PC**) and *Ulva* sp. (**UL**). The macroalgae were included in diets at 4% of dry matter in a randomized complete block design, with two diets tested. Low fiber diet (**LFD**), consisting of 39% corn silage, 49% corn grain, and 12% soybean expeller (CP: 10.5 %, NDF: 19.8 %, ADF: 8.5 %, DMD: 80.1 %, ME: 2.88 Mcal/kg DM); high fiber diet (**HFD**), consisting of 48% corn silage and 52% mixed pasture (CP: 14.1%, NDF: 35.7 %, ADF: 17.1%, DMD: 77.1%, ME: 2.78 Mcal/kg DM). All treatments, including the control (without algae), were incubated at 39°C in an *in vitro* gas production system. Methane production was measured at 4, 8, 12, 24, 30 and 48 hours of fermentation. Total CH₄ production was expressed as the cumulative production over the 48-hour incubation period. For each experimental period, three incubation bottles per treatment were used, and the experiment was replicated across three independent periods.

In the LFD, no significant effects were observed with the algae treatments at the applied doses. However, in the HFD, UPL and HL significantly reduced CH₄ production by approximately 12% and 16%, respectively, compared to the control (58.18 and 55.03 vs. 65.75 ml/g degraded OM), without affecting organic matter degradability (*p* > 0.05).

In conclusion, wild sources of UPL and HL proved effective in mitigating enteric CH₄ emissions in HFD. However, further studies are essential to elucidate the mechanisms underlying this reduction. Future research should also evaluate the long-term effects of macroalgae supplementation on animal performance, rumen fermentation, and the scalability of this approach in commercial livestock systems.

214 Patents in methane emission reduction technologies for ruminants, with a special emphasis on Mexico

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Methane is the second most significant greenhouse gas (**GHG**) contributing to global warming, behind carbon dioxide. Over the past 15 years, methane concentrations in the atmosphere have increased so quickly that they may endanger current attempts to lower carbon dioxide emissions. A systematic search was conducted across the Derwent Innovation (Clarivate™) and SIGA-IMPI databases (Mexican Institute of Industrial Property) to identify protected technologies within Mexico's jurisdiction. Of 23 identified patents (2000–2023), 11 are granted, 10 are pending, and 2 are invalid. Notably, 100% of applicants/owners are foreign entities, primarily from Japan, the USA, Switzerland, the UK, and Spain, with no domestic participation. Granted patents focus on two innovation pathways: Feed Additives: Allicin, phenolic compounds (naringin, neo-hesperidin), rhamnolipids, hop acids, bromoform, 3-nitrooxypropanol (**3-NOP**), and microbial consortia for microbiome modulation. Methodologies: Quantification protocols, microbiome-based selection of low-methane phenotypes, and methane reduction assessment systems in dairy cattle. Our findings reveal that feed additives dominate Mexico's patent landscape (78% of granted patents), aligning with global trends prioritizing scalable solutions for enteric methane abatement. The absence of Mexican applicants underscores a critical innovation gap, contrasting with nations enforcing stringent GHG policies (e.g., USA, Switzerland), where market-driven patenting activity correlates with regulatory frameworks. This study highlights the urgent need for policy interventions to incentivize domestic R&D and technology transfer in methane mitigation. Strengthening North-South collaborations could accelerate the adoption of context-specific solutions, bridging disparities in agricultural innovation capacity while advancing global climate targets.

216 Relationship of grazing and rumination duration with methane emission patterns in grazing cows fed concentrates

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Accurately predicting methane (CH₄) emissions from ruminants without knowing the dry matter intake is challenging. In previously published studies, emissions patterns determined in respiration chambers were successfully predicted using diurnal eating activity patterns. However, in many studies the association between total eating time and CH₄ emissions at a daily resolution was poor, suggesting the diurnal patterns should be considered if eating time is to be used to predict CH₄ emissions. We aimed to explore the relationship between the diurnal patterns of CH₄ emissions and the duration of grazing and rumination activities during the day to establish the potential of grazing duration and predict the daily CH₄ emissions in dairy cows. Methane emissions from 72 dairy cows, in two separate experiments, were measured for 5 weeks using GreenFeed units (C-Lock, Rapid City, South Dakota, USA) and data were reported as emissions in g/d for each spot sample. Eating and rumination time were continuously recorded for each cow using Nedap collars (Nedap, Groenlo, The Netherlands) and each activity was reported as min per 2-h-interval. The cows grazed ryegrass-based pasture *ad libitum* as the base diet and received concentrate supplements twice daily during milking (various levels of the same concentrate in Exp.1 and different types of concentrates in Exp. 2). Before data analyses, the eating and rumination time was averaged within cow across all days in each experiment for each 2-h-interval, while the CH₄ emissions were averaged by hour (as regularly processed) and then by 2-h-interval within cow to bring the CH₄ data to the same resolution level as the behaviour data. Only cows with CH₄ emission records in at least 50% of the 2-h-intervals were included in the data analysis (n =110). Pearson correlations of grazing and rumination duration with the corresponding CH₄ emissions were calculated between cows using a daily resolution level and within cow using the 2-h resolution level. The correlation between daily CH₄ emissions vs eating or rumination time were not significant when using data with a daily resolution. On the other hand, significant correlations were detected between eating duration and CH₄ emissions ($r = 0.48$), and between rumination time and CH₄ emissions ($r = -0.37$) using a 2 h resolution level. A mixed model that included the eating or rumination duration and 2-hour interval as a 3rd-degree polynomial as fixed effects and cow nested within the experiment as a random effect was used to predict the CH₄ emissions. Eating or rumination duration and 2-hour interval explained part of the variation in CH₄ emissions, but a larger proportion of the variation was associated with the individual variation. Prediction of CH₄ emissions when DMI is unknown, using eating or rumination duration requires a deeper characterisation of traits contributing to the individual variation.

219 How does using crop residues from garden markets in ruminant feeding affect enteric methane emissions?

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Improving feed intake is a key strategy for mitigating enteric methane emissions (**eCH₄**). Peri-urban livestock farmers in sub-Saharan Africa (**SSA**), use garden market crop residues. This study aimed to assess the impact of supplementation with sweet potato (*Ipomoea batatas*), cabbage (*Brassica oleracea*), green bean (*Phaseolus vulgaris*), and zucchini (*Cucurbita pepo* L.) leaves on intake, digestibility, and eCH₄ emissions in cattle. The study was conducted on five Sudanese Fulani zebu bulls, weighing 276.7 ± 33.16 kg and aged 71 months ± 1.18 years old, housed in individual boxes at the **CIRDES** (International Center for Research-Development on Livestock in the Subhumid Zone) experimental station in Burkina Faso. Five rations were tested: R1 (75% *Panicum maximum*, 25% *Ipomoea batatas* leaves), R2 (75% *P. maximum*, 25% *Brassica oleracea* leaves), control R0 (100% *P. maximum* hay), R3 (75% *P. maximum*, 25% *Phaseolus vulgaris* leaves) and R4 (75% *P. maximum*, 25% *Cucurbita pepo* L. leaves). They were served individually and daily to the animals at 3.5% of Body weight (**BW**) on a dry matter (**DM**) during the trials. Each trial lasted 26 days, consisting of 14 days of diet adaptation followed by 12 days of data collection. Five rations were tested from November 2024 to April 2025. During each trial, refusals and faeces were collected daily and weighed to calculate feed intake and apparent digestibility. The eCH₄ emissions were measured using the GreenFeed® system (ID: 252, C-Lock, Inc., USA) every four hours, to cover all hours of a day after 4 days of measurement. The results showed that the nutritional quality of rations differs according to the type of supplement used. The intake of mixed rations was higher than that of control R0. This intake was 18.88 ± 3.82 , 21.89 ± 3.36 , 24.88 ± 1.62 , 20.92 ± 3.71 and 22.27 ± 4.26 g DM/kg BW for R0, R1, R2, R3, and R4 respectively. The digestibility (0.53 ± 0.07) of R1 and R2 (0.50 ± 0.04) was higher than that of R0 (0.41 ± 0.12). In contrast, the digestibility values of R3 (0.41 ± 0.11) and R4 (0.41 ± 0.06) were similar to those of R0. Emissions of eCH₄ were 0.60 ± 0.07 , 0.70 ± 0.06 , 0.62 ± 0.03 , 0.60 ± 0.06 , and 0.51 ± 0.06 g/kg BW for rations R0, R1, R2, R3, and R4 respectively. There was no difference ($p > 0.05$) between the yields of R0 (33.01 ± 7.63 g/kg DM intake) and R1 (33.29 ± 5.20 g/kg DM intake). Rations R2, R3 and R4 induced reductions of 19%, 11%, and 28% respectively. The eCH₄ yields recorded in this study were close to the value of 0.68 ± 0.12 g/kg BW reported by Gbenou et al. (2022) but higher than the value of 23.3 g/kg DM intake by the IPCC (2019). The diversity of feed resources needs to be taken into account to improve national GHG inventories for the livestock sector in SSA.

224 Methane emission from horses fed two different rations

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Horses emits methane due to anaerobic fermentation of mainly fibers in the hindgut. In horses, starch, fat and protein are totally or partly digested and absorbed in the small intestine and would not contribute to the methane emission. Measurements of methane emission from horses are scarce. The enteric methane emission from horses is included in the national inventories and in Denmark it is assumed that 2.5% of the gross energy intake is lost as methane.

The aim of the study was to measure the methane emission from horses fed grass-based diets either supplemented with a fiber rich concentrate source in the form of sugar beet pulp (**SBP**) or a more starch rich concentrate source in the form of steam rolled peas (**PEA**).

Four 3-year-old Norwegian Fjord horse mares (body weight 386 ± 25 kg) were used in the experiment. The experimental design was a cross-over study where half of the horses in the first three weeks were fed either a diet where 30% of the net energy were from either SBP or PEA. The remaining 70% of the net energy was coming from haylage. In the next period of three weeks the horses switched diets. The methane emissions were measured by means of indirect calorimetry over the last three days in each period. During the measurements dry matter intake (**DMI**) was measured. The feed supply for the horses were divided into equal portions fed at 0930 and 1730 each day. Both SBP and PEA were soaked in water prior to feeding. The horses were gradually adapted to the stay in the chambers. The data was analyzed with PROC MIXED in SAS with diet and period as fixed effect and horse as random effect.

The DMI was not significantly different ($P=0.16$) between horses fed the two diets (SBP: 5.6 and PEA: 5.3 kg DM/day). The methane emission was 41 L/day for SBP and 57 L/day for PEA. Although the difference was at 16 l/day the difference was non-significant ($P=0.24$) as one horse on the PEA treatment produced 96 L CH₄/day. The CH₄ emission per kg DM were in average 7.3 and 10.7 L for SBP and PEA, respectively. On average 1.6% of gross energy intake was lost as methane for the SBP diet whereas 2.3% was lost for the PEA diet. The difference was non-significant ($P=0.20$), but the high emitting horse had significant influence on the result. The hydrogen emission per kg DM were significantly ($P=0.04$) higher for PEA (2.4 L/kg DM) compared to SBP (1.1 L/kg DM).

In conclusion, the methane emission per kg DMI did not differ between the two tested diets. Large individual differences in methane emission were measured. Further the data indicated that a loss of 2.5% of the gross energy as methane which is used in the Danish inventories may be overestimated.

226 Low methane and nitrogen emissions strategy of yak in response to alpine nutritional stress

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The yak (*Bos grunniens*), an indigenous herbivore raised at altitudes between 3,000 and 5,000 m above sea level, is closely linked to more than 40 ethnic communities. It plays a vital role in the ecological stability, livelihood security, socio-economic development, and ethnic cultural traditions in the Asian Highlands. They provide the highlanders with meat, milk, fibre, leather, and dung (fuel). They are also used for the transportation of goods, travelling, as well as ploughing, and hold importance in many religious and traditional ceremonies. In addition to the natural harsh environment, yaks as all the year-round grazing animals, have to suffer from malnutrition during the seven-month long and cold winter. After long-term natural selection processes, yaks have adapted excellently to the harsh conditions of the Asian highlands, and developed large lung and heart, low heart rate, heat production capacity in the cold season, genes related to hypoxic response, and insensitivity to hypoxic pulmonary vasoconstriction, thereby maintaining foetal haemoglobin throughout life. In response to the shortage of forage grass in the rangelands during the cold season, yaks have evolved to have a shorter tongue and weaker taste, which allows them to have a wider range of food choices and higher feeding efficiency, while allowing them to prefer forbs with high nutritional content. These animals have also harboured a unique rumen microbiota with a distinct maturation pattern, that has co-evolved with host metabolism. Furthermore, yaks have low nitrogen and energy requirements for maintenance, alongside the advantages of low methane (**CH₄**) emission and urinary nitrogen excretion, thereby obtaining 'low-carbon' and 'nitrogen-saving' traits, and thus shaping them to be environmentally friendly ruminant species. By measuring the CH₄ emissions of 173 yaks, we noted that the total yak population (~20 million) on the Asian highlands emit 550 thousand tons of CH₄ per year, which is 30% lower than the IPCC estimates (790 thousand tons per year). This review aims to provide an overview of the comprehensive adaptive strategies of yaks with particular emphasis on the low-carbon and nitrogen-saving metabolic strategies in response to the severe nutritional stress in the Asian Highlands. A better understanding of these strategies that yaks employ to adapt to the harsh environment could be used in improving their production, breeding and management, gaining benefits in ecosystem services, developing high-quality pastoralism, and a more resilient livelihood to climate change in the Asian highlands.

229 Effect of alpha- and beta-acid profiles of *Humulus lupulus* on in vitro rumen methane emission

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Ruminants contribute to greenhouse gas emissions, primarily through methane generated during ruminal fermentation. Research has shown that hop pellets and their extracted compounds have the potential to reduce methane emissions. This anti-methanogenic effect of hop is thought to be mainly due to the presence of alpha- and beta-acids, whose concentrations vary among hop varieties. This study investigates the methane reduction potential of hop pellets with varying concentrations of hop acids. Furthermore, it explores the underlying mechanisms driving the anti-methanogenic effects of hop. Pellets of four hop varieties with distinct alpha- and beta-acid profiles were selected for this study: Cascade ($\alpha+\beta=114.2$ g/kg DM, $\alpha/\beta=0.84$), Opal ($\alpha+\beta=120.0$ g/kg DM, $\alpha/\beta=1.45$), Golding ($\alpha+\beta=91.0$ g/kg DM, $\alpha/\beta=1.46$), and Magnum ($\alpha+\beta=213.5$ g/kg DM, $\alpha/\beta=1.89$). Batch experiments were conducted at inclusion levels of hop pellets at 0%, 5%, 10%, 15%, and 20% of substrate (DM basis). To investigate the mechanism underlying the anti-methanogenic effect, additional experiments were conducted at a 10% inclusion level for all varieties. These experiments were performed under two conditions: a CO₂ environment and a combined H₂+CO₂ (1:1) environment. The substrate consisted of grass silage, maize silage, and dairy concentrate (30:30:40, w/w/w). Rumen inocula were obtained from three rumen-fistulated dairy cows maintained on a basal diet of 50% maize silage and 50% wilted grass silage (DM basis), supplemented with straw and concentrate according to milk yield. Each experiment was repeated three times over three different days, with three replicates per day of incubation. Data were analyzed using mixed-model ANOVA, followed by Dunnett's post hoc test for mean separation. All hop varieties reduced ($P<0.05$) the absolute methane production ($\mu\text{mol/g DM}$), with the extent of reduction linked to their total acid content. Magnum showed the highest reduction starting at a 5% inclusion level, followed by Opal (5%), Cascade (10%), and Golding (10%). Hop inclusion also influenced rumen fermentation, as evidenced by the reduction in total volatile fatty acid (VFA) production. However, the reduction in total VFA production was lower than the reduction in absolute methane production. As such, the relative methane production (methane/total VFA, mol/mol) was reduced ($P<0.05$) with all four hop varieties at inclusion levels of 10% or higher. A linear model based on alpha- and beta-acid concentrations indicated that both acids contributed similarly to methane reduction, though beta-acids resulted in a greater decrease in total VFA production compared to alpha-acids. Interestingly, comparisons of incubations in a CO₂ vs. H₂+CO₂ environment revealed no direct inhibition of methanogens by hop pellets, except in the case of Magnum. These findings suggest that hop pellets, or their extracted compounds, could serve as promising feed supplements or additives for mitigating enteric methane emissions. However, caution is warranted in attributing these observed effects solely to these acids, as specific acid types were not studied, and other secondary compounds may also be involved. Further in vitro and in vivo studies are required to explore the impact of hops and their compounds on the digestibility of feed and its components.

230 Dairy cow phenotypes related to methane yield

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Dairy cows vary considerably in methane yield (CH_4/kg dry matter (**DM**) intake (**DMI**)), however, phenotypic traits related to methane yield are little explored although important for selection of cows with low emissions. Therefore, this study aimed to analyze how individual cow's level for methane yield relates to other phenotypic traits. Experimental treatments were, besides control, two methane-reducing additives: 3-nitrooxypropanol (**3-NOP**) and nitrate. A total of 186 first-lactation Danish Holstein cows were used in a 3×3 Latin square design, where cows entered the study individually 2-3 weeks after calving with each period lasting 21 days. A basal diet was based on (% of DM basis) 55 forage (mix of 50:50 maize:grass clover silage), 18 rape seed meal, 15 barley, 10 sugar beet, and 2 minerals and vitamins. Treatments were added to the basal diet at 60 mg 3-NOP/kg DM (Bovaer) or 8.5 g nitrate/kg DM (Silvair). The enteric gas emissions (CH_4 , CO_2 , H_2) were measured using two GreenFeed units (C-Lock Inc.), and cows were offered max 1.1 kg DM concentrate daily in the GreenFeed as bait. Ruminal content was collected using an oral-ruminal probe (Ruminator) to determine volatile fatty acid (**VFA**) concentrations. Feed intake was measured with the Insentec feed bins (Hokofarm Group). Cows were milked twice daily and sampled one day per week for analysis of milk composition. Data used for statistical analysis were obtained during the last week of the experimental periods for each individual cow. The random cow effects (**RCE**) were extracted using a mixed model in SAS and Pearson correlations were calculated using Proc Corr. The mixed model included Treatment (T, $n = 3$), Period (P, $n = 3$), $P \times T$, and Experimental week number ($n = 81$) as categorical fixed effects, and Cow ($n = 186$) as random effect. Methane yield RCE (g/kg DMI) were positively correlated to RCE for CO_2 emission (g/kg DMI , $r = 0.60$, $P < 0.01$), total VFA concentration ($r = 0.21$, $P < 0.01$), rumen molar proportion of acetic and butyric acids (0.29 and 0.28, respectively, $P < 0.01$) and the content of fat in the milk (g/kg , $r = 0.21$, $P < 0.01$), but showed negative correlations with the RCE for total DMI (kg/d , $r = -0.27$, $P < 0.01$), milk yield (kg/d , $r = -0.27$, $P < 0.01$), energy corrected milk yield (kg/d , $r = -0.25$, $P < 0.01$) and rumen molar proportion of propionic acid ($r = -0.37$, $P < 0.01$). In conclusion, some phenotypes like $\text{CO}_2/\text{kg DMI}$, milk yield, rumen VFA concentration and molar proportions of the main VFAs are correlated to the cow's methane yield. The negative correlations between RCE for methane yield and RCE for milk yield indicate that the present breeding towards higher milk production also results in cows with less methane yield.

232 Evaluation of post-harvest treatment on anti-methanogenic potential of *Asparagopsis taxiformis* when fed to dairy cows

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Asparagopsis taxiformis in freeze-dried form is an efficient inhibitor of ruminal methanogenesis due to the preservation of the bioactive component bromoform. However, freeze-drying is energy-intensive and can be difficult on a large scale. Recent studies have shown steeping the seaweed in oil is a promising alternative preservation method of bromoform. Hence, this study aimed to compare the methane-mitigating potential in dairy cows of *Asparagopsis taxiformis* subjected to 2 different post-harvest methods. During 3 experimental periods, 4 rumen cannulated lactating Danish Holstein cows received either 1) control diet without any seaweed (**CON**), 2) the same diet as CON but with the addition of freeze-dried *Asparagopsis taxiformis* (**FRE**; aimed bromoform content: 20 mg/kg dry matter (**DM**)), or 3) fresh *Asparagopsis taxiformis* biomass blended with fresh water and oil and subsequently stabilized with β -cyclodextrin (**BLEND**, aimed bromoform content: 20 mg/kg DM), resulting in one complete and one incomplete 3×3 Latin square. A similar amount of rapeseed oil (13 g/kg DM) was added to CON and FRE to compensate for the additional fatty acids added to the BLEND diet. Each period lasted 21 d and consisted of 14 d of adaptation, followed by 3 d of digesta sampling, and 4 d of gas exchange measurements using respiration chambers. The rumen was visually inspected for signs of damage such as rumen papilla atrophy and ulcers prior to the experiment and on d 17 in each period by rumen evacuation. Milk yield and feed intake were recorded daily. Blood samples were collected in each period on d 15 and 17. All parameters were statistically analysed using a mixed procedure of R. FRE significantly reduced DM intake (**DMI**) compared with CON (20.5 vs. 21.9 kg/d; $P < 0.01$), while both FRE (33.7 kg/d) and BLEND (33.9 kg/d) caused significant reductions in energy-corrected milk yield (**ECM**) compared with CON (35.9 kg/d; $P = 0.02$). Due to the changes in milk production and DMI, BLEND had a significantly lower feed conversion ratio (-7%; kg ECM/kg DMI) than CON. Daily methane production was significantly reduced on both *Asparagopsis* treatments (-30 and -26% on FRE and BLEND, respectively; $P = 0.02$), while methane yield (g CH₄/kg DMI) only tended to be reduced compared with CON ($P = 0.10$). The lack of significant differences in methane yield between *Asparagopsis* treatments and CON was caused by a large variation in cow responses. Hydrogen yield (g H₂/kg DMI) was significantly increased by 722 and 775% on FRE and BLEND, respectively ($P < 0.01$) compared with CON. Inspections during rumen evacuation and preliminary assessment of video recordings revealed that 2 out of 4 cows experienced minor ulcers and/or bleeding from the rumen wall when fed *Asparagopsis* treatments. In conclusion, both *Asparagopsis* products resulted in large numerical reductions in enteric methane emissions from dairy cows; however, concerns can be raised regarding reductions in feed intake, milk production, and ulcers in the rumen.

233 Dosing of bacteria-free rumen fluid isolated from a low emitter to high methane-emitting dairy cows

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During the last decade, a range of naturally occurring archaeal viruses targeting methanogens have been isolated and/or described using bioinformatics. The viruses attack the archaea in a host-specific way and thus modulate the microbial community. Under the assumption that low methane-emitting dairy cows have a higher population of archaeal viruses, this study aimed to investigate the methane-mitigating effect of intra-ruminal dosing of bacteria-free rumen fluid isolated from a low emitter to high methane-emitting dairy cows. Prior to the experiment, one low methane-emitting cow (12.9 g CH₄/kg dry matter intake (**DMI**)) and four high methane-emitting cows (19.4 ± 1.37 CH₄/kg DMI; average ± SD) were identified based on data from previous studies at our facility. All cows were rumen cannulated and fed the same total mixed ration throughout the experiment. The four high-emitting lactating Danish Holstein cows were included in a balanced 2 x 2 Latin square design receiving either **AUTO** (autoclaved centrifuged rumen fluid from low emitter cow) or **SWAP** (bacteria-free rumen fluid from low emitter cow) in the two experimental periods each lasting 17 d. The cows were placed in respiration chambers on d 14-17. The treatments (2 L of AUTO or 2 L of SWAP) were dosed directly into the rumen on d 14 when the cows entered the respiration chambers. The AUTO treatment was prepared by collecting rumen fluid from the low-emitting cow. The fluid was filtered and 3 times centrifuged (1X 1,000g and 2x 20,000g). Rumen fluid was made oxygen-free by bubbling with nitrogen gas and autoclaved. The SWAP-treatment was prepared on the same day as dosing to maintain the viability of viruses. Rumen fluid from the low emitter cow was filtered and centrifuged 3 times (1X 1,000g and 2x 20,000g) before being passed through a 0.45 µm polyethersulfone membrane filter to remove cell fragments. The bacteria-free rumen fluid was bubbled with nitrogen gas for 30 min. Both treatments were allowed to reach room temperature before dosing. All variables were statistically analysed using a mixed procedure of R. Milk production as kg energy corrected milk yield (**ECM**; 24.5 vs. 24.3 kg ECM/d for AUTO and SWAP, respectively) and dry matter intake (DMI; 21.4 vs. 20.9 kg DM/d for AUTO and SWAP, respectively) were similar between AUTO and SWAP. Daily methane production (g CH₄/kg DMI) was significantly greater for AUTO compared with SWAP (P=0.05; 417 vs. 398 g/d for AUTO and SWAP, respectively); however, when corrected for milk production and DMI, methane yield (g CH₄/kg DMI; 19.4 vs. 19.0 g CH₄/kg DMI for AUTO and SWAP, respectively) and intensity (g CH₄/kg ECM; 19.1 vs. 18.6 g CH₄/kg ECM for AUTO and SWAP, respectively) were similar between the treatments. In conclusion, intra-ruminal dosing of bacteria-free rumen fluid from a low methane-emitting cow to high emitters did not affect methane yield and intensity.

235 Effect of legume and enzymatic supplementation on growth, excreta moisture, and carbon footprint of broiler

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The goal of this study was to determine the effect of legume grains (**LG**) and enzymatic supplementation on growth performance, excreta dry matter content, and global warming potential (**GWP**) of broilers. A total of 480 one-day male Ross 308 broilers were distributed into 48 pens with 10 birds each. The pens were divided equally into the following treatments, **HN-STD** (high nutrient density diet with standard enzyme mix), **MN-STD** (moderate nutrient density diet with standard enzyme mix), **LG-0** (UK legume grains without enzyme mix), **LG-STD** (UK legume grains + standard enzyme mix), and **LG-SU** (UK legume grains + super dose of enzymes mix). The standard enzyme mix was 0.01% phytase and 0.01 % Signis (Xylooligosaccharide and xylanase) and the super dose enzyme mix was 0.05 % phytase and 0.015 % Signis. UK legume grains (a combination of lupin, faba bean and field pea) in LG diets replaced 50% of the soyabean meal in the control diet. All LG diets had moderated nutrient density. Bird weight, feed intake and feed conversion ratio were recorded on D10, D25, and D35. One bird/pen was sampled to on D35 to determine meat yield. Litter samples were collected for each pen on D35 and then analysed for dry matter content. Life cycle analysis was performed for each pen by calculating the GWP of the consumed feed then normalise that for 1 kg of meat yield. One-way anova was used to analyse the data. There was no significant difference between the experimental diets in D35 bird weigh, D0-D35 bird weight gain, D0-D35 feed intake, nor meat yield ($P>0.05$). The diets containing UK legume grains with either standard or super dose of enzyme did not significantly differ from MN-STD in feed conversion ratio. Soybean intake (g soybean meal/ g bird weight gain) and GWP (CO₂ equivalent/ 1kg of meat) was significantly reduced by LG diets compared to HN-STD ($P<0.05$). In conclusion, UK legume grains with enzymatic supplementation could replace half of soybean meal without affecting growth, excreta dry matter content, nor meat yield of broilers fed moderate nutrient density diets. Furthermore, this displacement reduced GWP of 1 kg of broiler meat by 34%.

241 Methane emissions on late gestation of beef heifers classified for residual feed intake

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Selecting replacement heifers based on residual feed intake (**RFI**) may help improve forage utilization and reduce methane emissions in cow-calf operations, which account for the highest forage consumption and methane emissions in beef production systems. Therefore, it is important to evaluate the impact of RFI on different stages and categories of the cow-calf herd. Our objective was to evaluate the effect of extreme RFI (low vs. high) on metabolic heat production (**MHP**) and enteric CH₄ emissions of first-caving heifers during the late gestation. Forty pregnant heifers were taken from an Angus herd of 72 heifers from INTA Naredo's (Buenos Aires Province, Argentina) herd, previously classified by RFI (i.e., 10-11 mo. old) using individual automatic feeder. During the trial, the heifers were fed a mixed ration with 80% oat silage and 20% protein-mineral concentrate in DM basis. Methane and MHP were measured by using an open-circuit gas quantification system (GreenFeed system; C-Lock). MHP was calculated using the following equation: $MHP = 3.866 \times O_2 + 1.2 \times CO_2 - 0.518 \times CH_4$. Additionally, the methane yield (**Ym**), which refers to the efficiency of feed utilization by the animal, was estimated by CH₄ emissions per unit of gross energy (**GE**) intake. The results indicated no significant effect of RFI on feed intake, CH₄ emissions, MHP, or Ym ($p > 0.05$). On average, feed intake was 4.65 ± 0.36 kg DM/animal for low RFI and 5.30 ± 0.38 kg DM/animal for high RFI. Methane emissions were 31.91 ± 7.2 g/kg DM, and the Ym was 8.87 ± 2.0 %GE. While, on average MHP was 23.57 ± 1.4 Mcal/day. Overall, methane emission (i.e., per animal, and in DM basis or GE basis) as well as MHP during the pre-calving period was relative high to early gestation or non-pregnant cows, but similar between low vs. high RFI heifers.

252 Developing emission factors and mitigation actions for enteric methane at different production systems in Indonesia

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Indonesia employs three primary beef cattle production systems: intensive, semi-intensive, and extensive. Each system utilizes distinct types of basal feeds, varying in quality and nutritional composition, which influence greenhouse gas (GHG) emissions, particularly enteric methane. This study aimed to determine appropriate emission factors (EF) for beef cattle under these production systems by evaluating feed intake, digestibility, and methane emissions associated with different basal diets and mitigation strategies. Experimental trials were conducted using native grass for semi-intensive and extensive systems, and cultivated grasses, such as Napier and Pakchong grass, as well as rice straw, for the intensive system. All diets were supplemented with concentrate. To mitigate methane emissions, several additives were tested: seaweed (*Eucheuma cottonii*) and *Gliricidia sepium* were incorporated into intensive system diets, *Leucaena leucocephala* into the extensive system, and solid decanter, a palm oil industry by-product, into the semi-intensive system. Methane emissions were measured using a laser methane detector. Results revealed that EFs for basal diets were 40.71, 42.74, and 46.41 kg CH₄/head/year for Napier grass, Pakchong grass, and rice straw, respectively. Native grass yielded EFs of 46.56 and 42.99 kg CH₄/head/year in semi-intensive and extensive systems. The use of mitigation agents showed variable impacts: *Gliricidia* increased EFs for Napier and rice straw diets to 46.54 and 49.99 kg CH₄/head/year, respectively, whereas *Eucheuma cottonii* reduced the EF of Pakchong grass to 36.84 kg CH₄/head/year. The inclusion of *Leucaena* and solid decanter with native grass lowered EFs to 41.43 and 46.16 kg CH₄/head/year, respectively. These findings underscore the significant influence of both feed type and mitigation agents on EFs. The variability observed highlights the importance of using accurate, locally derived data for national GHG inventories and mitigation strategies in the livestock sector.

256 Methane mitigation potential of native tropical forages from Mexico

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Enteric methane (**CH₄**) emissions from ruminant livestock contribute significantly to agricultural greenhouse gases, particularly in tropical regions where cattle diets are often based on low-quality forage. The Lacandon rainforest in Southern Mexico hosts a rich diversity of native forage species that could improve cattle diets and mitigate CH₄ emissions. Fifteen multipurpose forage species were selected based on their availability and importance in local livestock production systems. This study aimed to evaluate the nutritional quality, fermentation kinetics, and CH₄ mitigation potential of tropical forage species through a two-phase study conducted in Southern Mexico.

The first phase focused on screening 15 forage species for nutrient composition and CH₄ production potential, while the second phase investigated *in vitro* methane mitigation by incorporating selected species into mixed diets with tropical grasses. In the first phase, the 15 selected forage species were assessed for chemical composition, digestibility, and CH₄ emissions. Gas production was measured using a modified *in vitro* technique, with methane quantified by gas chromatography. Data were analyzed using a randomized block design, and treatment means were compared using the Tukey test ($\alpha = 0.05$). In the second phase, five species with promising CH₄ mitigation potential - *Spondias mombin*, *Acacia pennatula*, *Parmentiera aculeata*, *Brosimum alicastrum*, and *Bursera simaruba* - were mixed at two levels (15% and 30%) with *Pennisetum purpureum* grass. Parameters including crude protein (**CP**), fiber content, condensed tannins (**CT**), dry matter degradability, and CH₄ production were analyzed. Statistical analysis employed a randomized block design, with treatment means compared to the control (*Pennisetum purpureum*) using the Dunnett test ($\alpha = 0.05$).

In phase one, *Bursera simaruba* exhibited the lowest CH₄ production (9.08 mg CH₄/g degraded organic matter) and high CT content (200.1 g/kg DM), suggesting strong mitigation potential. Nutrient composition varied widely among species, with CP ranging from 99 to 289 g/kg DM and digestibility negatively correlated with content of secondary metabolites. In phase two, mixed diets with 30% *S. mombin* or *B. alicastrum* showed significantly higher dry matter degradability and reduced CH₄ emissions compared to the control (*Pennisetum purpureum*). Inclusion of *A. pennatula* at 30% slightly decreased CH₄ emissions, likely due to its high cyanogenic glycoside content. This study highlights the potential of integrating native tropical forage species into cattle diets to mitigate CH₄ emissions while improving nutritional quality. *Bursera simaruba* and *Spondias mombin* were particularly effective in mitigating CH₄ emissions due to their secondary metabolite profiles (200.1 and 9.9 g/kg DM of CT, respectively). These findings support the adoption of locally available forage resources as a sustainable strategy for enhancing livestock production systems in tropical regions.

262 In vitro evaluation of *Rosa rugosa* Thunb. on ruminal fermentation and methane production

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Greenhouse gas emissions have gained more attention as a significant environmental concern that demands innovative and sustainable solutions, such as the use of agro-industrial byproducts. *Rugosa* rose (*Rosa rugosa* Thunb.) fruit pomace (**RFP**) is a byproduct of juice-producing companies with promising benefits in ruminant nutrition due to its bioactive compounds and nutrient profile. This study aimed to investigate the effect of fresh and ensiled RFP on in vitro ruminal fermentation, methane production, and biohydrogenation processes. The evaluation had three treatment groups and was conducted in a short-term incubation system using 40 ml of buffered ruminal fluid from a Polish Holstein Friesian cow under an anaerobic condition for 24 hours at 39 °C. Treatment groups had 200 mg samples of grass silage as a control (n=15; 5 bottles × 3 consecutive incubations), fresh RFP (**RFPF**; n=15), and ensiled RFP (**RFPS**; n=15). After the incubation, pH was measured using a pH meter, in vitro dry matter digestibility was carried out, while ammonia (**NH₃**) concentrations (Nessler methods), volatile fatty acid profiles, and methane (**CH₄**) production were determined using gas chromatography. All data were subjected to a one-way analysis of variance and analyzed using R Studio (V 2022). The RFPF and RFPS increased ruminal pH (p<0.05) from 6.02 to 6.47 and 6.28 and decreased NH₃ (p<0.05) by 31.2% and 26.8%, respectively. Total VFA concentrations and IVDMD decreased (p<0.05) by 28% and 10% in the RFPF group and by 15% and 7% in the RFPS group, respectively, compared to the control group. Furthermore, propionate increased (p<0.05) by 5.5-15.4%, and acetate decreased (p<0.05) by 37.7-21.5%, respectively in RFPF and RFPS. Moreover, favorable changes in the fatty acid profile were observed, indicating reduced biohydrogenation. Specifically, C18:1 cis 9, C18:2 cis 9 trans 11, and C18:3 n-3 increased (p<0.05), while C18:0 decreased (p<0.05) as a result of RFPF and RFPS treatments. Additionally, including both RFPF and RFPS reduced in vitro methane production (p<0.05) by 23.4% and 18.3%, respectively. This study concluded that incorporating RFP into dairy cows' diets in in vitro conditions can promote environmental sustainability.

269 Enteric methane mitigation in dairy heifers: the effect of bovaer in different doses

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Bovaer, including the active component 3-nitrooxypropanol (**3-NOP**), is a commercially available methane mitigating feed additive approved for dairy cows and pregnant heifers in the EU. The objective of this study was to investigate the dose-dependent effects of Bovaer on enteric methane emission and production performance of dairy heifers. A total of 32 Danish Holstein heifers were enrolled in a randomized block design from 3.5 to 20 months of age. Heifers were blocked according to initial body weight and randomly allocated to 1 of 4 dietary treatments reflecting different doses of 3-NOP. The treatments were BOV0, BOV75, BOV150, and BOV225 (0, 75, 150, and 225 mg 3-NOP/kg DM, respectively). The Bovaer was included in a total mixed ration formulated to meet the requirements of a heifer with an expected growth of 800 g/day. From 3.5 to 5.5 months of age, the heifers were fed a diet with 60:40 forage to concentrate ratio, whereas heifers from 5.5 months of age were fed a diet with 95:5 forage to concentrate ratio (on a dry matter basis). The heifers were kept in 2 deep litter pens with 16 animals in each pen. Animals within each pen were allocated to 1 of 4 Insentec feed bins containing the assigned dietary treatment. Heifers were weighed every two weeks to monitor growth. Gas exchanges were consecutively measured at 4, 8, 12, 16, and 20 months of age in 4 of the 8 blocks of heifers using respiration chambers. Data from respiration chamber measurements at age 4 and 8 months are presented in this abstract. Data were analyzed using a mixed model including the fixed effect of treatment, age, block, and the interaction between treatment and age. Bodyweight and dry matter intake were not different between treatments. At 4 and 8 months of age, heifers averaged ($\pm$ standard error of mean) 142 and 249 $\pm$ 2.6 kg of bodyweight, respectively, and had a dry matter intake of 4.3 and 5.3 $\pm$ 0.15 kg/day, respectively. At both ages, methane production (g/day) and methane yield (g/kg of dry matter intake) was reduced linearly ($P < 0.05$) by increasing doses of Bovaer. Methane yield was reduced by 13, 26, and 39% in heifers at 4 months of age fed BOV75, BOV150, and BOV225, respectively, relative to BOV0 (19.4 g methane/kg of dry matter intake). At 8 months of age, methane yield was reduced by 7, 16, and 19% in heifers fed BOV75, BOV150, and BOV225, respectively, relative to BOV0 (23.3 g methane/kg of dry matter intake). These results document methane mitigating effects of Bovaer when fed to dairy heifers at varying ages, however, the trial is ongoing, thus the results are preliminary. Additional data representing even longer duration of Bovaer supplementation and progressing developmental stages of the heifers will elucidate if the documented effects of Bovaer are persistent throughout the rearing period and will document potential associated effects on animal performance (e.g. dry matter intake and growth).

271 A sustainable livestock approach: Chilean grape marc as a feed ingredient to mitigate environmental impacts

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Grape marc (**GM**), is a relevant by-product of Chilean winemaking industry. Due to its complex matrix, which consists of fiber, protein, minerals, and phenolic compounds, it has been explored as a potential feed additive to mitigate the environmental impact of ruminants. The objective of this study was to evaluate the effects of dry GM (20% of dry matter [**DM**]) inclusion as a forage substitute in a perennial ryegrass (*Lolium perenne* L.) based diet with a 70:30 forage-to-concentrate ratio on methane (**CH₄**) and ammonia nitrogen (**NH₃-N**) production under *in vitro* conditions. Data were obtained from two independent experiments: a batch culture (three runs, three replicates per treatment, and three sampling times: 6, 12 and 24 h, using a total of 54 bottles) and a semi-continuous fermentation culture using a Rusitec apparatus (two runs, each with 10 days of adaptation followed by 6 days of sampling). Additionally, a quantitative PCR analysis was conducted in the Rusitec culture to assess the effects of GM inclusion on total methanogen populations. The data from the batch study were analyzed using a one-way ANOVA, with GM inclusion, as fixed effect, and the run as random effect. In contrast, the results from Rusitec were examined with a repeated measures ANOVA, accounting for GM inclusion, incubation period, day, and the interaction between treatment and day as fixed effects, while run was considered a random effect. The GM inclusion reduced the *in vitro* DM disappearance ($p < 0.001$) by 4.5% in the batch study; however, this effect was not observed in the Rusitec study ($p > 0.05$). The batch study revealed that GM inclusion reduced ($p < 0.001$) CH₄ production (mg/g DM incubated) and yield (mg/g DM digested) by 15% and 14%, respectively, while decreasing ($p < 0.001$) NH₃-N (mg/dL) production by 58%. Similarly, the Rusitec study confirmed that GM inclusion (20% DM) reduced ($p < 0.001$) CH₄ yield by 15% and daily NH₃-N production by 25%. The qPCR analysis revealed that GM inclusion decreased total methanogen populations by sevenfold ($p < 0.001$) throughout the entire experimental period. The reductions in CH₄ yield observed in these experiments are consistent with previous findings using different GM varieties from Australia, Italy and Spain, which suggest that the decrease in ruminal methanogenesis associated with GM, is the result of the combined effects of lignin, fat, and tannin contents, which impair rumen fermentation and subsequent CH₄ formation. However, based on the findings of this study, it is possible that the impact of GM inclusion on enteric CH₄ production includes a direct influence on methanogen populations. It remains to be elucidated whether these effects are a result of the secondary metabolites or the fats present in the GM. Conclusion: *In vitro* studies demonstrate that GM inclusion (20% DM) into high-forage ruminant diets significantly decreases CH₄ production and NH₃-N release, reduction that could be associated with a substantial decline in ruminal methanogen populations, while maintaining favorable ruminal fermentation parameters.

272 Dietary strategies for rumen methane mitigation: a meta-analysis of *in vitro* studies (2016–2024)

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The prohibition of certain chemicals and ionophores in ruminant feed has spurred interest among researchers in exploring natural alternatives to mitigate enteric methane (CH₄) production from livestock. *In vitro* systems provide a practical platform for evaluating such strategies. Despite numerous global studies, including in Chile, challenges remain in identifying effective and sustainable alternatives to address this environmental issue. The objective of this meta-analysis of Chilean *in vitro* studies was to assess the potential of various natural dietary strategies for mitigating enteric CH₄ emissions *in vivo*. Data from 12 studies (24 h batch incubation) published in peer-reviewed journals and conference proceedings between 2016 and 2024 were included (n = 36 treatment means). Methane mitigation strategies, including activated carbon; algae (*Agarophyton chilensis*, *Macrocystis pyrifera* and *Lessonia spicata*); aquatic plant (*Potamogeton pusillus*); fungi (*Pleurotus ostreatus*); by-products such as crude palm oil and different varieties of grape marc (GM, *Vitis vinifera*); cyanogenic glycosides (linamarin); essential oils (*Origanum vulgare*) and their main components (carvacrol and eugenol); polyphenolic extracts (*Pinus radiata*); saponins (*Quillaja saponaria*); and tannins (*Schinopsis balansae*), were categorized as feed additives or supplements based on their type, chemical composition, and dietary concentration. The CH₄ production [mg/g dry matter (DM) incubated], varied greatly across studies; therefore, the relative mean difference (as a percentage of the control mean) was used as the effect size in subsequent analyses. A random-effects model analyzed CH₄ productions relative to controls, outliers exceeding three standard deviations were excluded. A heterogeneity analysis was performed to assess variability among studies, and publication bias was evaluated (Egger's test). The results indicated low variability (I² = 23.5%; Q = 45.7, P = 0.128), with no evidence of publication bias (P = 0.891). The model estimated variability in true effects (τ² = 32.1), highlighting differences in the efficacy of mitigation strategies. Several additives or supplements reduced (P < 0.05) *in vitro* CH₄ production. Effective CH₄ mitigation strategies included *P. pusillus* at 40% DM (−46.7%), *P. ostreatus* at 6% DM (−35.7%), sweet GM at 25% DM (−25.3%), *M. pyrifera* at 10% DM (−22.3%), and fermented GM at 20% DM (−20.2%). Other effective strategies included País GM at 20% DM (−18.3%), tannins at 4% DM (−15.8%), essential oils at 0.07% DM (−10.8%), and polyphenolic extracts at 6% DM (−7.32%). Conversely, the other strategies did not affect CH₄ production in this meta-analysis. The evaluated dietary strategies showed consistent effects, suggesting a generalized positive impact on the environment, highlighting the potential of natural additives and supplements to mitigate enteric CH₄ emissions. However, *in vivo* applicability varies considerably, with some strategies (e.g., GM at moderate inclusion levels) being more practical than others requiring implausible inclusion levels (e.g., *P. pusillus*). In addition, most studies reported CH₄ production per gram of incubated DM. A more accurate approach is to express CH₄ production per gram of digested DM, as digestibility directly affects emissions and values can vary significantly depending on the chosen metric. Therefore, further research is needed to ensure the balance between the dietary strategy efficacy and its feasibility, before field validation.

273 Dried distiller's grains supplementation to reduce enteric methane emissions and nitrogen excretion from grazing-beef heifers

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Dry distiller's grains with solubles (**DDGS**) are extensively used in livestock feed and could potentially influence methane (**CH₄**) emissions and improve nitrogen use efficiency. Twenty-four Angus pasture-fed heifers (281±23 kg LW) were used to evaluate the enteric methane mitigation potential and nitrogen excretion by corn-based DDGS supplementation. Three weeks before the supplementation period, heifers were allocated to alfalfa (*Medicago sativa*) strip-graze paddocks (3 heifers/paddock, 4 paddocks/treatment). Each paddock was assigned randomly to 1 of 2 treatments: 1) control, grazing without supplementation (**CTRL**); 2) grazing with 1% LW of DDGS supplementation (**DGS**). Animals from DGS were supplemented once daily at 8:00 for 70 days. Animals were weighed periodically for average daily gain (**ADG**) gain determination. After 14 days of starting the treatments and during the whole experiment, enteric CH₄ emissions were measured using a GreenFeed unit (C-Lock Inc.). Two heifers per paddock (subsampling) were used for blood, rumen fluid, urine, and fecal sampling over two sampling periods (days 33 and 67). In each period, rumen fluid and blood were collected once for VFA and nitrogen determination. Urine and fecal samples (grab sampling) were collected over 3 days at the following time points (pool): 18:00, 24:00 (1st day), 6:00, 12:00, 21:00 (2nd day), and 3:00, 9:00, 15:00 (3rd day) and frozen at -20°C for nitrogen and dry matter intake determinations. Feed chemical composition and animal samples' lab analysis are still underway. So far, alfalfa chemical composition was 31.8% DM, 48.6% NDF, 39.4% ADF, 16.0% CP, and corn-based DDGS chemical composition was 91.9% DM, 9.5% ADF, 28.2% CP, 10.1% total lipids. Animal performance data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with the pen as the experimental unit. Heifers supplemented with DDGS showed a greater ($P=0.0002$) average daily gain than CTRL (1.09 vs. 0.67 kg/day, 0.035 SEM). Although CH₄ data are still under analysis, it appears that DDGS supplementation would reduce CH₄ production. These results will be presented at the conference and will allow us to conclude regarding DDGS supplementation as a strategy to reduce CH₄ intensity in pasture-fed heifers. Dry matter intake and nitrogen excretion lab analysis are in progress.

278 *Camelina sativa* cake reduces enteric methane emissions in heifers by suppressing methanogen without compromising digestibility

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This study evaluated the effect of *Camelina sativa* L. cake (**CSc**), a C18:3 n-3 source on enteric methane emission, ammonia production, dry matter intake (**DMI**), and ruminal fermentation. In a crossover design, eight Polish Holstein-Friesian heifers (369 ± 31 kg body weight) were assigned to two dietary groups (CON and EXP; n = 4). Each period lasted 26 days, comprising a 21-day adaptation phase and a 5-day sampling phase (4 days of gas analysis and 1 day of rumen fluid collection). The basal diet provided once daily at 10:00 a.m. consisted of a TMR including grass silage (386 g/kg of DM), maize silage (208 g/kg of DM), beet pulp (119 g/kg of DM), rapeseed meal (133 g/kg of DM), meadow hay (119 g/kg of DM), and a mineral blend (35 g/kg of DM). The EXP heifers received 1kg of CSc per day. An additional 1 kg of rapeseed meal was provided to the CON group (day/heifer) to balance the protein content. Feed intake was recorded individually throughout the experiment. Four respiration chambers (SPA System, Ltd., Wroclaw, Poland) were used to monitor CO₂, CH₄, and NH₃ emissions. On day 26 of each experimental period, rumen fluid samples (500 mL) were collected via stomach tubing (Ruminator, Profs Products, Wittybreut, Germany) at 1:00 p.m., 3 hours after feeding. Immediately after collection, the pH of the rumen fluid was measured using a portable pH meter, and two subsamples were prepared to determine volatile fatty acids (**VFA**) and ammonia nitrogen (**NH₃-N**). To analyze microbiota abundance (qPCR, copy numbers of *mcrA*, and *ssr* - 16S rRNA genes), rumen fluid was aliquoted (1 mL), snap-frozen in liquid nitrogen, and stored at -80°C. The EXP diet reduced methane and ammonia emissions (g/d) by 11% and 30%, respectively. Methane mitigation was accompanied by a decrease in the methanogen population, as indicated by a reduction in the *mcrA/ssr* gene copy ratio from 5.39% (CON) to 2.00% (EXP). A higher incidence of *Prevotella spp.* may be significant, as their association with elevated propionic acid concentrations could reduce hydrogen availability in the rumen, thereby limiting methanogenesis. Importantly, a statistically significant improvement was also observed in the digestibility of dry matter and total protein, which indicates a positive effect on overall feed efficiency and nutrient utilization.

285 Effect of methane-reducing additives on ruminal headspace gas composition in milk-fed dairy calves

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The objective of this study was to quantify the effect of methane reducing additives on ruminal headspace gas composition in dairy calves at slaughter. Twenty-four calves were divided in six blocks of four calves each. Within block, each calf was assigned to one of four treatments, which represented different approaches for early life imprinting of the rumen microbiome using methane mitigating feed additives. Three treatments were based on daily oral dose of either water (Control), Bovaer in water solution, or Nitrate in water solution. The dosing started from birth, and it was administered two hours after the morning milk feeding. The fourth treatment was the same as Control, but Bovaer-enriched concentrate pellets were provided ad libitum from birth. Calves on the other three treatments had ad libitum access to non-Bovaer-enriched concentrate pellets, and all calves had ad libitum access to hay and water. Calves were weaned from milk at eight weeks of life. The oral doses of Bovaer and Nitrate were proportional to the body weight of the calves, aiming at a dose of 5 mg of 3-nitrooxypropanol and 120 mg nitrate per kg of body weight. The Bovaer-enriched pellets included 300 mg 3-nitrooxypropanol per kg of dry matter. On the day of weaning, calves received the last oral dose of the treatment, and calves on Bovaer-enriched pellets were moved to regular ones. After 24 hours, the calves were exsanguinated, and the rumen was isolated with zip ties before being extracted from the body. Samples were collected in duplicates by puncturing the dorsal rumen sac. The syringe was flushed two times with headspace gas before a 10mL sample was drawn and transferred to evacuated Exetainers. Finally, the rumen was weighed with and without content, and a subsample of rumen content was used for dry matter determination. The gas content of the Exetainers was analysed with gas chromatography for concentrations of methane, hydrogen, carbon dioxide, oxygen, and nitrogen. The data was analysed with a Generalized Linear Model assuming gamma distributed residuals in R software, using treatment as fixed effect. The estimated means and standard errors were 24.7 ± 5.05 , 21.3 ± 3.98 , 23.0 ± 4.30 , and $14.1 \pm 2.88\%$ for methane concentration ($P = 0.23$); 0.29 ± 0.19 , 0.080 ± 0.046 , 0.15 ± 0.087 , and $6.88 \pm 3.99\%$ for hydrogen concentration ($P < 0.001$) for Control, Bovaer, Nitrate and Bovaer-pellets, respectively. No differences between treatments were observed for carbon dioxide, oxygen, and nitrogen concentration (66.7 ± 5.76 , 0.68 ± 0.196 , and $9.96 \pm 2.90\%$, mean across treatments $\pm$ minimum standard error). Finally, the rumen content of dry matter was added to the model as a linear covariate, with this model there was a negative correlation between rumen dry matter and content of oxygen ($P < 0.01$) and nitrogen ($P = 0.02$), respectively. In conclusion, rumen headspace gas of calves fed Bovaer-enriched pellets had greater hydrogen concentration (and numerically lower methane concentrations) than calves on Control, possibly indicating a reduced methanogenesis. Oxygen and nitrogen concentration in the headspace gas samples indicate negligible contamination with atmospheric air.

288 Anaerobic fungal MALDITOF database construction: advancing identification and understanding of their role in ruminant methanogenesis

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Anaerobic fungi (**AF**) inhabit the rumen and alimentary tracts of most herbivorous animals, where they play a crucial role in lignocellulose degradation. They also contribute indirectly to ruminal methanogenesis through their extracellular relationship with methanogens (archaea) to produce methane gas, a significant source of energy loss and greenhouse gas. A deeper understanding of these processes requires the isolation, cultivation, and identification of more AF species/strains, many of which remain poorly studied and underexplored. However, routine methodologies, including standard subculturing, microscopy, and gene sequencing, are often costly, time-consuming, and labour-intensive, limiting their widespread application. Currently, **MALDI-TOFMS** (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) has been widely used for the rapid identification of common bacteria, yeast, and aerobic fungus with no application with AF. Also, its self-construct library lacks a database and a stated standard sample preparation for building a database for AF. To support AF identification via MALDI_TOF, this study focused on the isolation of AF from sheep rumen fluid. The rumen fluid was inoculated into Orpin's grass medium supplemented with antibiotics and incubated for 5 days at 39°C to serve as enrichment media. Subsequently, this was used to inoculate Orpin's cellobiose agar via roll tubing incorporating antibiotics and vitamins. Single colonies were picked under strict anaerobic conditions and the process was repeated 3-4 consecutive cycles to ensure purity. Pure isolates were obtained and 5 distinct ones were used to start the development of a customized spectral database accounting for fungal growth phases. Two growth time points were selected: 4/5 days corresponding to peak growth in the broth and two days thereafter. However, challenges arose during sample preparation, particularly in sample collection and the selection of an appropriate lysing buffer. To address these issues, this experiment therefore modified the sample preparation in two distinct ways. First, liquid enrichment media were employed instead of colonies on agar plates due to difficulties in cultivating AF on agar plates. Additionally, higher centrifugation speed (14000g) and extended duration (10mins) were used in the protocol. Second, both bacterial lysing solutions (lysate 1 and lysate 2) and a lysing solution optimized for filamentous fungus (MSA05) were utilized for the 5 isolates to enhance the quality of the resulting spectra. It was observed that the 5 isolates investigated gave the best spectral at 4/5 days when MSA05 was used. Although the study is still ongoing, it has confirmed the successful cultivation and isolation of pure AF isolates. The preliminary findings indicate that a tailored MALDI-TOF database can substantially enhance the rapid identification of AF, offering a practical alternative to conventional molecular methods. Incorporating multiple growth phases into the database strengthens its robustness, capturing the metabolic diversity of AF. To ensure validation and accuracy, MALDI-TOF data will be soon associated with molecular and microscopy outcomes. This advancement improves the study of rumen microbiomes and provides tools for optimizing feed efficiency while reducing methane emissions. Future research should focus on expanding the database to encompass additional AF species/strains and validating its effectiveness across diverse sample sets.

290 Plant extract greatly reduces *in-vitro* methane production in exotic but not indigenous cattle

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Reducing methane emissions from enteric fermentation is a global priority. Phytochemicals like tannins, saponins and flavonoids which are in abundance in many tropical plants are reported to have anti-methanogenic effects. This study was therefore conducted to investigate the phytochemical composition and anti-methanogenic potential of some medicinal plants from semi-arid part of Nigeria. Two experiments were conducted. In the first experiment, four plants: *Guiera senegalensis*, *Leptadenia hastata*, *Combretum micranthum*, and *Diospyros mespiliformis* were extracted via ultra-sonication using water, absolute ethanol, and 70% ethanol to obtain twelve different extracts. The extracts were randomly used as treatments (50 g kg⁻¹ inclusion) in an *in-vitro* gas production experiment where Timothy hay (100 mg) was incubated in syringes using rumen fluid from slaughtered cattle in Malaysia, with 30 µL of chloroform used as a positive control. Three separate runs were conducted for the experiment with each treatment replicated thrice in each run. The yield and phytochemical composition of the extracts were determined. Total gas production, and methane production were measured while short chain fatty acid was predicted. Based on the result obtained in the first experiment where absolute ethanol extract of *C. micranthum* greatly reduced methane production, a second experiment was conducted using rumen fluid from cattle in Nigeria to determine the methane reduction effect of ethanol extract of *C. micranthum* and *C. micranthum* leaf powder. Two runs of the experiment were conducted with treatments having six replicates. Total gas and methane production were measured. Results of extract yield and phytochemical composition in Experiment 1 were described using percentages and means while gas production data in both Experiments 1 and 2 was analysed using Analysis of Variance. Absolute ethanol produced the least extract yield (46.9 g kg⁻¹) in all the medicinal plants, while water yielded the highest extracts in *L. hastata* (244 g kg⁻¹), *G. senegalensis* (167 g kg⁻¹) and *C. micranthum* (144 g kg⁻¹), while 70% aqueous ethanol yielded highest extract in *D. mespiliformis* (238 g kg⁻¹). Extracts of *D. mespiliformis* had the highest amount of phenols (58.3 g kg⁻¹, SE=0.920), tannins (38.5 g kg⁻¹, SE=1.015) and flavonoids (151.7 g kg⁻¹, SE=1.079). There was a significant ($P<0.001$) effect of treatments on total gas and methane production. Absolute ethanol and 70% aqueous ethanol extracts of *L. hastata* as well as 70% aqueous ethanol extract of *C. micranthum* increased the total amount of gas produced. Chloroform and absolute ethanol extract of *C. micranthum* significantly ($P<0.001$) reduced methane production by 87% and 88% respectively while water extract of *C. micranthum*, absolute ethanol and 70% aqueous ethanol extracts of *L. hastata* increased methane production. Predicted short-chain fatty acids were significantly ($P<0.001$) reduced by both chloroform and absolute ethanol extract of *C. micranthum*. In the second experiment, absolute ethanol extract of *C. micranthum* and *C. micranthum* leaf powder both reduced methane production by approximately 14%. It was concluded that *C. micranthum* has anti-methanogenic potential and warrants further investigation to determine its mode of action and effect in *in-vivo* trial.

291 Methane emission in beef bulls classified by residual feed intake

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Residual feed intake (**RFI**) has become a key parameter for estimating energetic efficiency in beef cattle, independent of animal growth and body size. Residual feed intake is a trait with moderate heritability, and animals with low RFI may have lower methane production.

The aim of our study was to evaluate the relationship between RFI and methane production in young beef Hereford bulls. A total of 28 young Hereford bulls (average initial live weight = 223 ± 42 kg) from a herd of 38 bulls classified by RFI were grouped into low RFI (**LRFI**) and high RFI (**HRFI**) categories to measure CH₄ emissions. The bulls were fed a ration consisting of 78% oat silage and 22% concentrate mix (including wheat mill, corn grain, soybean meal, sunflower meal, and minerals) for 104 days (14 days for adaptation, 60 days for RFI evaluation, and 30 days for methane production evaluation). Methane emissions were measured using an open-circuit gas quantification system (GreenFeed system; C-Lock®).

Liveweight gain (**LWG**) did not differ between bulls with high vs. low RFI ($P = 0.18$). Feed intake tended ($P = 0.08$) to be lower for bulls with LRFI than those with HRFI, and feed efficiency was higher for LRFI bulls ($P < 0.01$). Methane emissions per bull per day ($P = 0.11$) and per kg of LWG ($P = 0.93$) did not differ between LRFI and HRFI bulls. However, methane emissions per kg of dry matter intake were lower for HRFI bulls compared to LRFI bulls ($P < 0.01$).

In summary, LRFI bulls had a similar impact on methane emissions per head as well as per unit of liveweight gain. Future research is needed to evaluate a broader range of genetic lines to identify animals with low methane emission.

292 Methane emissions response to different levels of corn supplementation to cattle fed a forage-based diet

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For several decades, different strategies have been developed to reduce enteric methane (CH_4) emissions from ruminants. One of the most effective strategies for ruminants on forage-based diets is increasing concentrates in the diet. Studies showed that this inclusion modifies rumen fermentation pathways, reducing methane emissions and improving productive performance. The extent to which methane reduction becomes significant with the inclusion of concentrate, as well as its impact depending on the animals' age, remains uncertain. This study aimed to evaluate methane emission responses in two beef cattle categories fed forage-based diets with increasing levels of corn grain. Forty-eight Hereford beef cattle were used, comprising two categories: 24 calves (10 $\pm$ months, 236 $\pm$ 16 kg of BW), and 24 steers (22 $\pm$ months, 466 $\pm$ 19 kg of BW). Animals were blocked by BW and distributed across three feeding pens with four automatic feeders each. For eighty days they were fed *ad libitum* using a forage-based diet of alfalfa plus *orchardgrass* haylage and increasing levels of corn grain as treatments (T): T0 (0%), T20 (20%), T40 (40%) and T60 (60%). The individuals' daily gains (ADG) and dry matter intakes (DMI) were measured during the experiment. The SF₆ technique adapted for long-term (5-day) periods was used to measure absolute enteric CH_4 emissions (g/day) from each animal. Response variables included DMI (kg DM/day), ADG (kg BW/day), CH_4 (g/day), CH_4 /DMI (g/kg DM) and CH_4 /ADG (g/kg BW gain). Data were analyzed using linear mixed models in R software, with significance set at $p < 0.05$. DMI and ADG showed a linear increase ($p < 0.05$) with the increasing levels of corn supplementation, being 0.031 vs 0.048 kg/day and 0.013 vs 0.006 kg/day for the calf and steer categories respectively. Absolute CH_4 emissions differed significantly by category through all levels of concentrate ($p < 0.05$). Steers showed a quadratic reduction with a tipping point at 20-40%, while calves showed a linear increase across treatments. Both categories showed a negative linear relationship ($p < 0.05$) for the emission efficiency (CH_4 /DMI), with a reduction of -0.10 and -0.07 g/kg per % corn grain for steers and calves, respectively. The same occurs for emission intensity (CH_4 /ADG) which decreased linearly ($p < 0.05$) in both categories with the inclusion of concentrate, being 1.9 and 2.5 g/kg, for the steer and calf categories, respectively. Despite contrasting trends in absolute emissions for steers and calves, both categories demonstrated significant improvements in emission efficiency and intensity with higher levels of concentrate. Additionally, productive variables such as DMI and ADG increased consistently in both steers and calves. These results highlight the potential of concentrate inclusion to enhance productive performance and mitigate methane emission efficiency in beef cattle, while emphasizing the differing responses in absolute emissions based on age and physiological stage.

295 Dynamics of enteric methane production of steers responding to an oil-encapsulated bromoform Investigational Veterinary Product

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This project investigated the dynamics of enteric methane production from steers before, during, and after the inclusion of an oil-encapsulated bromoform (**eCHBr₃**; Rumin8 Pty Ltd, Australia) in their diet. Twenty Angus crossbred steers (348 ± 6.2 kg [mean $\pm$ SE] liveweight) were fed a 55% tempered barley feedlot transition ration *ad libitum* and spent two 9-day measurement phases in open-circuit respiration chambers to measure methane (**CH₄**). The first phase included 2 days of Baseline measurements with no eCHBr₃ treatments, followed by a 7-day Onset period where they were fed either a High (40 mg/kg DM) or Low (20 mg/kg DM) dose of eCHBr₃ mixed into the feed. After an additional 10 days fed treatments in pens, the steers returned to the respiration chambers for a second 9-day phase, including a 2-day Pre-Withdrawal period with treatment diets and a 7-day Withdrawal period without eCHBr₃.

The mean daily CH₄ production for the High treatment was $116 (\pm 3.7)$, $15.0 (\pm 3.2)$, $6.47 (\pm 2.5)$, and $101 (\pm 5.5)$ g CH₄/day for Baseline, Onset, Pre-Withdrawal, and Withdrawal, respectively, and $112 (\pm 4.1)$, $44.5 (\pm 5.4)$, $69.6 (\pm 7.7)$, and $118 (\pm 3.3)$ g CH₄/day for the Low treatment. A mixed-effects model was fitted using the 'lme4' package in R, with fixed effects for Period, Treatment, and their interaction, and a random effect for steers. The results indicated a significant decline in CH₄ production from Baseline to Onset ($P < 0.001$) and a significant increase from Pre-Withdrawal to Withdrawal ($P < 0.001$). However, there was an average increase of 25 g CH₄/day between Onset and Pre-Withdrawal ($P = 0.004$) in the Low treatment, but no difference in the High treatment. This suggests steers on the Low treatment increased daily CH₄ production during the 10 days between these periods when methane measurements were not recorded.

To describe the relative changes in CH₄ production in the High treatment, a four-parameter logistic model was fitted for the response of CH₄ (g/day) during the Onset phase (Baseline and Onset) and separately for the Withdrawal phase (Pre-withdrawal and Withdrawal). The model fits a curve with an asymptote at the start (A-asymptote) and end (B-asymptote) of the phase, with an incline or decline representing the rate of change between the upper and lower CH₄ values. These results indicate it took approximately 37 h (1.5 days) for CH₄ emissions to decrease by 95% of the difference between the A- and B-asymptotes during the Onset phase, whereas it took 75 h (3.1 days) for CH₄ to increase by 95% during the Withdrawal phase. The inflection points (Onset = 20 h, Withdrawal = 42 h) also suggest that, on average, the increase in CH₄ during Withdrawal took twice as long as the decline during Onset.

In conclusion, daily inclusion of 40 mg/kg DM of an oil-encapsulated bromoform in the diet of feedlot steers resulted in a 95% reduction in daily CH₄ production within 1.5 days, sustained over 19 days before recovering to baseline emissions within 3 days of treatment withdrawal.

298 Chicory mitigates methane emissions in dairy cows without compromising milk yield compared to perennial ryegrass

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This study aimed to investigate the effects of feeding chicory compared to perennial ryegrass on methane (**CH₄**) emission, feed intake, and lactation performance in dairy cows. A total of 32 Holstein-Friesian dairy cows (130 ± 41.9 days in milk; mean ± standard deviation) were enrolled in a randomised complete block design. The experiment lasted 28 days with 14 days for adaptation and 14 days for data and sample collection. Cows were blocked according to parity, days in milk, and milk yield, and randomly assigned to their dietary treatments with chicory or ryegrass being the sole roughage source. Chicory and ryegrass were harvested once daily and provided in feed bins that automatically recorded individual feed intake. Animals received concentrates (1.8 kg dry matter per day) in equal portions twice daily during milking. In addition, a maximum of 2.2 kg dry matter per day of concentrate was provided via the GreenFeed system that was used to measure emissions of CO₂, CH₄, and H₂ through the data collection period. Data were analysed using the MIXED procedure of the SAS software with diet considered as fixed factor and block as random factor. Feeding chicory did not affect daily milk yield and fat- and protein-corrected milk (**FPCM**) yield of dairy cows, but led to a 7% lower ($P = 0.02$) roughage intake, an 11% lower ($P < 0.01$) milk fat content, and a 52% lower ($P < 0.01$) milk urea content compared to ryegrass. Chicory feeding was associated with a 24% lower ($P < 0.01$) CH₄ production (g/d), and a 22% lower ($P < 0.01$) CH₄ intensity (g/kg FPCM) compared to ryegrass. No dietary effect was observed on the number of GreenFeed visits, nor did visit frequency influence estimated CH₄ production. In comparison with ryegrass, feeding chicory did not affect CO₂ emissions, whereas H₂ production and intensity were both 75% greater ($P < 0.01$). In conclusion, feeding chicory markedly decreased CH₄ emissions and milk urea content in dairy cows compared to perennial ryegrass. While milk yield was unaffected, milk fat content was reduced. Chicory offers a viable alternative to perennial ryegrass for mitigating methane emissions while maintaining milk production.

299 Eco-milking: a breakthrough-solution for methane-reduction and dairy productivity-enhancement in climate-resilient agriculture using marine-algae feed supplements.

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Methane emissions from livestock are a major contributor to global greenhouse gas levels, while the dairy industry continues to grapple with improving milk yield and quality under the pressure of sustainable farming demands. Addressing both challenges requires a solution that not only enhances productivity but also reduces environmental impact. This study presents a novel nutritional supplement derived from a unique blend of marine algae - brown (Phaeophyceae), red (Rhodophyceae), green (Chlorophyceae), and blue-green algae (Cyanophyceae). These algae are naturally rich in minerals, trace elements, and bioactive compounds, but their inherent polysaccharides are largely indigestible to ruminants. To unlock their potential, a proprietary extraction process was developed to break down these polysaccharides into bioavailable monomers. The resulting formulation delivers sulphated polysaccharides and essential minerals in a readily absorbable form. Acting as both a nutrient source and a prebiotic, the supplement supports rumen health by improving fermentation efficiency, increasing volatile fatty acid (VFA) production, and reducing carbon dioxide in the gut. Since CO₂ supports methanogenic microbes, its reduction leads to lower methane emissions. This dual action enhances animal productivity while curbing greenhouse gas output.

Field trials validate the product's effectiveness. In Australia, a year-long study was conducted on a family-run organic dairy farm in Victoria with 320 Jersey cows. Each cow received 10 ml of the supplement daily via a feed-line doser. The results showed a 30% increase in milk yield compared to the control group and a reduction in bulk milk somatic cell count (**BMCC**) from 300,000 to 250,000, indicating improved milk quality. A separate study in Nepal covered three different dairy farms. The trial groups showed milk yield increases of 0.32, 0.15, and 0.12 kg/lit respectively, compared to control groups which saw gains of 0.26, 0.12, and 0.09 kg/lit. This translated into a 23.07%, 25.00%, and 33.33% increase in milk yield, with an average improvement of 27.13% across the farms. These gains reflect enhanced nutrient absorption and better feed efficiency enabled by the supplement. A third trial focused on methane mitigation through in vitro analysis. In control samples without the supplement, gas production began within 12 hours, and methane levels reached 4800 ppmv. However, in samples treated with the supplement (branded VEPMAXX), gas production was delayed to 17 hours, and no methane was detected for concentrations between 0.3 and 0.15 ml/kg TMR. Methane was only seen at a minimal 1000 ppmv at lower concentrations (below 0.075 ml/kg TMR). These findings show strong potential for methane suppression without compromising fermentation, suggesting that the product improves rumen function while cutting greenhouse gas emissions.

In summary, this algae-based solution integrates marine science with practical dairy nutrition. It not only boosts milk output and quality but also reduces methane emissions through targeted microbiota modulation and improved feed efficiency. With consistent results across geographies and herd types, the supplement offers a scalable, science-backed tool for climate-smart dairy farming that meets both productivity and sustainability goals.

303 Effect of ensiled grass pulp on dairy cow dry matter intake, milk and methane production

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Economic pressures have intensified rearing practices, necessitating increased use of concentrate feeds. However, agricultural land suitable for traditional protein crops is scarce, making Norway reliant on protein import. In contrast, Norway's climatic conditions favours both large yields and high nutritive quality of grasslands. Extracting green protein concentrate from grassland crops through green biorefineries could alleviate import dependency. However, this process leaves a considerable volume of fibrous pulp, necessitating a high-value side-stream utilization. Previous experiments testing grass pulp as forage to ruminants have showed promising results. As green biorefinery is novel to Norway, exploration under local conditions with commonly used grassland species and varieties is required. Improved forage quality is well-proven as a methane abating measure in ruminant productions. How grass pulp depleted of water-soluble nutrients will influence enteric methane emissions is less studied. The current study investigated the effect of replacing regular whole-plant grass-silage with ensiled pulp in dairy cow diet on productivity and enteric methane emissions. An 18-days data collection period was preceded by a 1-week covariate period followed by a 3-week adjustment period. Control cows ($n=20$) were offered whole-plant grass-silage, while cows in treatment group ($n=20$) had this replaced in full by ensiled pulp. The two forages were harvested simultaneously from the same grassland, a ley with a mixture of *Phleum pratense*, *Lolium pratense*, and *Trifolium pratense*. Concentrate rations were calculated individually based on pre-experimental performance and *ad libitum* forage intake. Production performance and body-condition score were recorded in an AMS unit. Methane emissions were estimated using a GreenFeed concentrate feeder. Feed intake was recorded by access-controlled feed bunks and concentrate feeders. A mixed effect ANOVA was performed using SAS, with treatment (pulp or grass-silage), parity (primiparous or multiparous), lactation stage (early, mid, or late), and experimental day (continuous) as fixed effects and cow as random. Corresponding pre-response variables were included as covariate. Preliminary results show a significantly lower mean daily dry matter intake (**DMI**) in the pulp group. With this gap mainly explained by difference in silage intake, forage constituted 65 and 62% of total DMI in grass-silage and pulp groups, respectively. Differences in milk yield (kg/cow/day) between pulp and grass-silage were not statistically detectable, however the pulp group tended towards higher energy-corrected milk yield (kg **ECM**/cow/day). This tendency was predominantly due to a significant increase in milk fat yield in the pulp group. No difference between groups on change in body condition score was observed, indicating improved feed efficiency in the pulp group. Relative to DMI, methane yield (g/kg DMI) was significantly higher for cows fed pulp silage. However, this increase was compensated for by the group's reduced feed intake. Thus, no effect was seen neither on absolute enteric methane production (g/cow/day), relative to performance (g/kg milk yield), nor intensity (g/kg ECM). Grass pulp replacing whole-plant grass-silage can maintain cow productivity. Despite an increase in methane yield, this was compensated for by treatment group's improved feed efficiency, making both production and intensity similar for both treatments.

304 Dynamics of enteric methane emissions during the wet season in Zebu cattle in Sub-Saharan Africa

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Livestock in sub-Saharan Africa is indexed as a major emitter of greenhouse gases, particularly enteric methane (**eCH₄**), when these emissions are reported per unit of product and/or feed intake. In this region, rangeland fodder is the main feed resource for livestock, especially in wet season. The aim of this study was to measure the dynamic of eCH₄ emissions from cattles according to the growth stages of wet season rangeland fodder: vegetative stage (**VeS**), reproductive stage (**ReS**) and mature stage (**MaS**). To this end, ten Sudanese Fulani zebu bulls (52 ± 1.2 months, 195 ± 14.6 kg in average initially) were fed green rangeland fodder of wet season. The experiment lasted 112 days split into 14 days of adaptation to the fodder and 98 days of data collection (21 days for VeS, 56 days for ReS, and 21 days for MaS, successively). The rangeland fodder consisted primarily of *Andropogon gayanus* Kunth. During the experiment, the cattles were divided into two batches of 5 each. Batch 1 was fed ad libitum at 4.5% of body weight (**BW**) dry matter (**DM**) basis (**4.5ING**) and batch 2 at the standard 2.5% of BW (**2.5ING**). The voluntary intake, digestibility, average daily gain (**ADG**), feed conversion ratio (**FCR**), and eCH₄ (GreenFeed® system) were measured for each animal per day per fodder growth stage and per offered level. Fodder growth stage and offered level influenced intake in bulls. The DM intake (g/kg BW) decreased progressively from 33 ± 7.7 in VeS to 26 ± 4.3 in MaS ($P < 0.001$) with the highest values for 4.5ING ($P < 0.001$). Only the growth stage had an impact on apparent digestibility. The DM digestibility was 0.74 ± 0.03 in VeS and 0.65 ± 0.07 in MaS ($P < 0.001$). The ADG and FCR were influenced by fodder growth stage and offered level. The ADG was significantly lower (294 ± 17.2 g/d) and FCR significantly higher (20 ± 1.1) in MaS. The ADG was 592.3 ± 18.1 and 353 ± 15.6 g/d for 4.5ING and 2.5ING, respectively ($P < 0.001$). The FCR was 13 ± 0.9 and 16 ± 1.0 for these respective offered levels ($P = 0.048$). Low eCH₄ yield (17 ± 0.9 g/kg DMI) and intensity (137 ± 10.2 g/kg ADG) were obtained in VeS ($P < 0.001$). Fodder allowance had no effect on eCH₄ yield ($P > 0.05$) but on eCH₄ intensity, for which average values of 213 ± 12.1 and 298 ± 14.7 g/kg ADG were obtained for 4.5ING and 2.5ING, respectively ($P < 0.001$). This study revealed that intake, digestibility, ADG, FCR, and eCH₄ yield and intensity are influenced by rangeland fodder growth in wet season. The fodder allowance does not influence digestibility and eCH₄ yield.

307 Tier-2 methane emission factor for pregnant alpacas grazing cultivated pastures in the Peruvian Andes

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Alpaca farming at the high-altitude Peruvian Andes is an indigenous livestock system, yet their greenhouse gas (**GHG**) contributions for inventory purposes remain underexplored. This study aimed to assess the impact of alpaca variety (Huacaya vs. Suri) and period (Transition vs. Wet) on the estimation of the Tier-2 emission factor (**EFs**; kg of CH₄ head⁻¹year⁻¹) in late-gestation female alpacas grazed rotationally on cultivated pastures. This study was conducted in Cusco, Peru (13° 55' 1.9" S, 71° 37' 14.7" W) at 4,141 m.a.s.l. Eight Huacaya (56.9 ± 4.29 kg LW) and six Suri (59.4 ± 4.29 kg LW) adult females were kept on a cultivated mixed pasture dominated by perennial ryegrass (68%), legumes (lucerne and clovers) and herbs (plantain and chicory) constituting 16% each, respectively. The first period (26 days) took place in November 2021, at the end of the dry season ('Transition'), with the females in their 8–9-month of gestation. Period 2 started in January 2022 (10–11-month of gestation), at the beginning of the rainy season ('Wet'). Experimental paddocks were set up on rotational grazing (60 days resting period) and area of grazing strips adjusted to guarantee an *ad-libitum* daily herbage allowance. Dry matter intake (**DMI**; g d⁻¹) was assessed via total faecal collection over five days and *in vitro* DM digestibility. Gross energy content (**GE**; MJ kg DM⁻¹) of the consumed diet was estimated using an empirical equation from chemical composition of the grazed herbage. Enteric CH₄ emissions were measured using the sulphur hexafluoride (**SF₆**) gas-tracer technique. Enteric Tier-2 EFs were computed from the GE intake (**GEI**) and the CH₄ conversion factors (CH₄/GEI; *Y_m*, %), both obtained in this study, according to guidelines in The Intergovernmental Panel on Climate Change (**IPCC**). Data were analyzed using a repeated measures model, with alpaca variety and experimental period as fixed effects, and alpaca within variety as random. This study demonstrated that experimental period (aligned with two late gestation stages) had a stronger influence than alpaca variety on feed intake and CH₄ emissions. Tier-2 EF decreased from 11.7 up to 8.61 kg of CH₄ head⁻¹year⁻¹ from the Transition to the Wet period, respectively (*P* = 0.041). Average Tier-2 EF for the entire late-gestation (8–11-month) was estimated to be 10.2 ± 2.57 kg of CH₄ head⁻¹year⁻¹. The last overestimated in 28 % the current IPCC fixed Tier-1 EF value for alpacas of 8.0 kg of CH₄ head⁻¹year⁻¹ (liveweight = 65 kg). Discrepancies on EFs between estimated Tier-2 and Tier-1 values are indicative for the context of this study and extrapolations should be taken with caution. One limitation is the short length of experimental periods. Also, IPCC Tier-2 equation does not account for liveweight losses (as was observed in Transition period). Despite drawbacks, the utilisation of Tier-2 EFs should be preferred and in the future improved (aiming for Tier-3 calculations). More studies under more real husbandry conditions (extensive) are required to help policymakers undertaking better decisions towards enhancing sustainability of traditional alpaca production. This underscores the need for developing EFs addressing specific production settings towards refining GHG inventories.

311 Breeding/selection low-methane forages: a strategy to reduce enteric methane emissions in the Global South

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Agri-food systems contribute about 25% of global greenhouse gas emissions, with methane (CH_4) emissions from livestock as the largest source. Consequently, reducing emissions from livestock, particularly enteric CH_4 , is a key strategy to mitigate climate change, and several technologies and approaches are being explored by researchers to this end. One of the mitigation approaches, key for livestock systems in the Global South (GS), is to identify anti-methanogenic compounds (AMCs) and introduce them into high-yielding, drought tolerant, and high nutritive value forage and fodder species/cultivars, to develop feed sources that will potentially lower enteric CH_4 emissions. To develop and deploy forage and fodder cultivars containing significant levels of AMCs that lower CH_4 emission in the GS, a collaborative research project is being carried out by three CGIAR centres (the Alliance Bioversity-CIAT, ILRI, and ICARDA) that hold the greatest forage and fodder biodiversity. The genetic diversity contained in the Centre's Genebanks, is expected to contribute to the discovery and selection of AMCs that can be introgressed into widely cultivated varieties, or even for direct deployment by farmers. Screening these materials, coupled with genomic and metabolomic analyses, will allow the identification of genetic markers associated with low methane production traits and AMCs in readily available germplasm. Part of the study aims to integrate field evaluation, genomics, metabolomics, and advanced plant breeding (including gene-editing) techniques to develop climate resilient high-quality forage and fodder cultivars that are adapted to diverse agroecological zones and livestock systems in the GS and beyond, and will result in low methane-emission livestock systems. To date the collaborating centres have assembled over 400 widely diverse genotypes of forage and fodder species originating from genebank collections and breeding programs that are being field-evaluated and assessed for agronomic performance. The genotypes are also being screened *in vitro* for methane production to help understand the amount, and variation, of methane emissions from the standard material prior to the development and deployment of novel forages containing AMCs. In addition, plant tissue samples from each genotype are being (have been) collected to be analysed for nutritive value using near infrared spectrophotometry (NIRS), and for genomic and metabolomic characterization. Finally, protocol optimization is underway for gene editing, using *Urochloa humidicola* forage grass, where it may be necessary to enrich widely utilized forage cultivars with AMCs via a gene editing approach. Two candidate genes, *bmr6* and *FatB*, have been identified that are involved in the metabolic pathways associated with digestion and nutrient absorption. The *bmr6* gene encodes the enzyme cinnamyl alcohol dehydrogenase (CAD), which plays a crucial role in the lignin biosynthesis pathway, and the *FatB* gene encodes an acyl-ACP thioesterase involved in fatty acid biosynthesis and altered rumen fermentation. The practice and utilization of gene editing targets Latin American countries where there are existing regulations for the application of gene-edited crops in agriculture. The transfer and use of products can only be possible in countries with an approved regulatory framework. Progress on developing and deploying these novel forages and fodder will be reported.

312 Effect of chemical fractions of dairy cow's diet on mitigating efficacy of 3-nitrooxypropanol in vitro

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Enteric methane emissions from cattle contribute to global warming. The feed additive 3-nitrooxypropanol (**3-NOP**), an inhibitor of methyl-coenzyme M reductase in methanogens, has already demonstrated a significant methane reduction potential. However, the mitigating effect of 3-NOP may be influenced by the chemical fractions of the basal diet. This study aims to investigate the effect of the dietary chemical composition on the effectiveness of 3-NOP in vitro and explore if the known interaction from in vivo research can also be observed in vitro. In the first in vitro batch experiment, the effect of 3-NOP on relative CH₄ production (methane/total volatile fatty acid, mol/mol) was evaluated after 24 hours at a dose of 0.760 mg/gDM, supplemented in 25 substrate combinations (5 forage and 5 concentrate mixtures) under standardised in vitro conditions and fermentation protocols. The applied dose was calculated based on in vivo additions and the different ratio of substrate to volume of rumen fluid between in vitro and in vivo conditions. Mixed rumen inoculum was obtained from three rumen-fistulated dairy cows maintained on a basal diet. All diet combinations had a forage-to-concentrate ratio of 65:35. The 5 forage mixtures consisted of pressed beet pulp (10%) and a varying grass silage to maize silage ratio (90%) of 0:100, 25:75, 50:50, 75:25 and 100:0. To broaden the range of the chemical fractions, each forage mixture was combined with 5 concentrates, resulting in the same net energy for lactation and rumen fermentable organic matter, but different chemical compositions and origin of those chemical fractions. The experimental design resulted in highly correlated covariates, which prevented fitting multivariate models. Therefore, models only included an overall mean and a single chemical variable (mean-centered). Average, minimum and maximum values (mg/gDM substrate) of the predicting chemical variables were: 281, 268 and 294 for NDF_{forage}; 71.1, 40.0 and 103 for crude ash; 97.0, 0.00 and 197 for starch_{forage}. 3-NOP reduced the relative methane production ($P < 0.001$) when supplemented with all combinations. Individually, NDF content of the forage, starch content of the forage and crude ash content affected the mitigating effect of 3-NOP, expressed as the relative mean difference (%) to the control. A 1% (10 mg/gDM) increase in substrate NDF_{forage} and crude ash content from their mean, decreased the efficacy of 3-NOP by 8.62%-units (model estimate: $-42.7 + 0.862 \times (\text{NDF}_{\text{forage}} - 281)$; $P < 0.05$) and 2.73%-units (model estimate: $-42.7 + 0.273 \times (\text{crude ash} - 71.1)$; $P < 0.05$), respectively. A 1% (10 mg/gDM) increase in substrate starch_{forage} content from its mean, increased the efficacy of 3-NOP by 0.742%-units (model estimate: $-42.7 - 0.0742 \times (\text{starch}_{\text{forage}} - 97.0)$; $P < 0.05$). So, the efficacy of 3-NOP is also influenced in vitro by the dietary chemical composition as observed in vivo. We are exploring whether multiple covariates can be included to refine predictive models by further varying the variation of feed components and ratios in follow-up experiments. Further analysis will also be conducted to quantitatively compare the variation in the mitigating effect of 3-NOP observed in vitro with the variation associated with the basal diet in vivo.

315 Impact of maize supplementation on performance and methane emission in fattening heifers fed high-quality forage

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In temperate regions, fattening cattle graze winter annual pastures (i.e., oats, ryegrass, rye, etc.), which may have excessive crude protein concentration (**CP**) and low energy required for finishing. This imbalance can be corrected by grain supplementation (i.e., maize grain and others). Besides that, energy supplementation has been proposed as a strategy to reduce methane (**CH₄**) emissions in beef cattle. Therefore, the aim of our study was to evaluate the effect of ground maize supplementation on CH₄ emissions, DM intake and liveweight gain (**LWG**) of beef heifers fed oat silage. Twenty-seven Angus heifers (initial liveweight (LW) = 244.07 ± 30.56 kg; age = 14 mo.) housed under similar environmental conditions were assigned to a completely randomized design. Experimental heifers were allotted to the following dietary treatments: 1) non-supplementation (**0GM**), 2) supplemented at 0.9% of LW of ground maize (**0,9GM**), and 3) supplemented at 1.8% of LW of ground maize (**1.8GM**) per animal per day on as fed basis. The experimental period consisted of an adaptation interval of 15 d (based on previous studies) and a 15 d to collection interval to measure (CH₄). Oat silage was fed *ad libitum* with automatic feeders (Hook®, Venado Tuerto, Buenos Aires, Argentina) and GM levels were individually fed with an automatic feeder (Suplementar®, Jesus María, Córdoba, Argentina). Daily CH₄ emission was individually collected using an open-circuit gas quantification system (**OCGQS**; Greenfeed system; C-Lock Inc., USA). The OCGQS individually delivered a protein-mineral concentrate (**PMC**; CP, 32%) formulated to correct the nutrients of the basal diet. Total, silage, GM, and protein-mineral concentrate intake as well as CH₄ emission was determined for each heifer. From the results, final LW ($P = 0.41$) and LWG ($P = 0.42$) did not increase in response to GM supplementation level ($P > 0.05$). Final LW was 328 ± 2.87, 330 ± 3.01 and 334 ± 3.09 kg for 0, 0.9 and 1.8% supplementation level, respectively. Average daily gain was 1.26, 1.28, and 1.34 kg of LW/d (SEM = 0.04) for 0, 0.9, and 1.8% maize supplementation, respectively. Methane emission per animal ($P = 0.65$) and per kg of LWG ($P=0.18$) did not differ among treatments. Intensity of methane emission was 142, 143, and 126 g/kg LWG for 0, 0.9, and 1.8% maize supplementation. It was concluded that substitution of high-quality forage by maize ground maize neither improved average daily gain, nor reduced total and intensity of methane emission in fattening beef heifers.

320 Impact of co-product supplementation on the carbon footprint of cattle raised on tropical pastures

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Global livestock farming has advanced in the adoption of technologies in search of greater efficiency and sustainability. Production systems based on pastures are subject to climate conditions and the consequent seasonality of forage production and nutritive value. A widely adopted technological alternative, especially in Brazil, is the supplementation of animals diets with energy or protein feed during critical periods of low forage availability. Many co-products of the agroindustry have high nutritional value for ruminants, including Dry Distillery Grains (**DDGS**) from the corn ethanol production, which has high protein content and a low carbon footprint. DDGS supplementation can directly reduce methanogenesis by providing highly digestible protein and favouring the ruminal fermentation process, as well as reducing total methane emissions by allowing better animal performance and shortening the production cycle. This project was conducted at the Faculty of Agricultural and Veterinary Sciences of UNESP, Brazil, with the objective of evaluating the carbon footprint ($\text{CO}_2\text{eq}^{-1}$ kg of live weight) of grazing beef cattle receiving four levels of DDGS supplementation. Sixty F1 Angus x Nellore bulls, in the rearing phase, with an initial body weight of 280 ± 4 kg, were used. The animals were distributed in a completely randomized design, in 4 treatments and 3 replicates (paddocks) of *Urochloa brizantha* Palisade grass. The treatments consisted of different levels of protein supplementation with DDGS: 0.00, 0.25, 0.50 and 0.75% of live weight. Supplement was administered in groups. The palisade grass pastures were managed in a continuous grazing system with variable stocking rate, using additional animals to maintain the forage height between 25 and 35 cm. To determine animal performance, the testers animals were weighed at the beginning and end of the experimental period, after a 14-hour fasting period. The average daily gain (**ADG**) was calculated from the difference between the initial and final fasting weight, divided by the experimental period of 112 days. To calculate the carbon footprint, in CO_2eq per kilogram of live weight, the Embrapa Low Carbon Livestock Calculator was used, based on the IPCC Tier 2 model (IPCC, 2019), which follows the FAO/LEAP guidelines (FAO, 2016), meets the requirements of ISO 14.044 (ISO, 2006) and the ICVCalc food library (Matsuura et al., 2022). The experimental units were the animals and the individual data were analyzed using Tukey's test at 5% probability in the statistical program R. Total dry matter intake was estimated and increased linearly ($p < 0.001$) with increasing supplementation, as did ADG ($p < 0.001$) with averages of 0.549, 0.755, 0.970 and $1.164 \text{ kg}^{-1} \text{ day}^{-1}$. Forage intake was estimated and remained unchanged ($p = 0.329$), as did stocking rates ($p = 0.442$). The model results indicate a potential 8.5% reduction in the carbon footprint of animals receiving 0.75% of live weight of supplementation, resulting in emissions of 4.409, 4.315, 4.100 and $4.035 \text{ kg of CO}_2 \text{ eq}^{-1} \text{ kg LW}$. It is concluded that protein supplementation is an efficient nutritional strategy to increase the performance of F1 Angus x Nellore bulls raised on tropical pasture in the dry-rainy transition season, contributing to the reduction of the product's carbon footprint.

321 Altitude and harvest day affects yield, nutritive value, and carbon footprint of Guatemala grass

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The goal of the study was to determine the effect of altitude (midland and lowland) and harvest day (90 days, 120 days, or 150 days of planting) on growth performance, nutritional value, *in vitro* ruminal fermentation kinetics, and methane (**CH₄**) production of Guatemala grass (*Tripsacum laxum*). The experiment was laid out in a factorial arrangement of two altitudes and three harvesting days in a randomized complete block design with three replications. Agronomic data (plant height, number of tillers per plant, number of leaves per plant, number of nodes per plant, and internode length), yield of nutrients (dry matter (**DM**), crude protein (**CP**), and metabolizable energy (**ME**)), nutritive value (CP, ME, potential intake of DM of CP intake, and potential ME intake) and CH₄ production were recorded. Increasing harvest day significantly increased biomass and nutrient yields regardless of the altitude ($P < 0.05$). The nutritive value of Guatemala grass decreased significantly as the grass age increased to 150 days ($P < 0.05$) without any change in CH₄ production (5.6 ml CH₄/g DM degraded; $P > 0.05$). Increasing the harvest day to 120 days would be recommended to improve the land productivity of Guatemala grass in both low land and mid land altitudes, thereby providing more nutrients to livestock for improved meat and milk production. A key advantage of Guatemala grass is that its CH₄ output remains unchanged across different agroecological zones, demonstrating its adaptability and potential for sustainable livestock production.

325 Catalogue of solutions for mitigation of greenhouse gas emissions in livestock and crop farms

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There is a growing body of evidence about effective options for greenhouse emission reduction in agriculture. Existing scientific information about mitigation options, however, is poorly accessible and usable to final users: farmers and advisors. Moreover, the number of mitigation options is large, the level of evidence and technical readiness vary among solutions and sectors, and effects often depend on the specific farm situation and way of implementation. Our aim, therefore, was to develop a catalogue of greenhouse gas mitigation options for European crop and livestock farms with science-based information about their effects, as part of the European ClieNFarms project. First, an inventory of mitigation options was made based on 54 papers and databases, complying with the criteria: i) potential reduction of total net greenhouse gas emission based on a life cycle assessment approach, ii) effective on European dairy, beef, sheep, pig or arable crop farms, iii) tangible action realized within farm gate boundaries, iv) evidence of effect in ≥ 2 peer-reviewed scientific articles, and v) technical readiness level 7-9. Second, a database was compiled with information per mitigation option: farmer's action, applicability, effects on greenhouse gas emissions and organic carbon stocks expressed on a 6-point Likert Scale, variation in effects, and explanations of the mechanisms of effects and potential causes of variation. Effects on emissions and soil organic carbon stocks were derived from about 350 scientific articles, of which nearly half were meta-analyses. If results of articles concerned single-source emissions, the effect on total cradle to farm-gate emission was estimated using the breakdown of emission intensity in the FAO GLEAM 3 dashboard (livestock) or UK benchmarks for feed wheat (arable crops), and augmented by expert judgment. Multiple expert sessions and factsheet reviews were conducted to verify the database contents. The initial inventory resulted in a total of 63 mitigation options related to animal management, feeding and nutrition, manure storage and treatment, pasture management, soil and crop management, fertilization, and energy use and production. However, due to insufficient literature or a lack of conclusive evidence, the number mitigation options was reduced to less than 50. Only few papers were found using Life Cycle Assessment, particularly for sheep and pig systems. Most papers concerned feeding and nutrition and soil and crop management, followed by fertilization and pasture management. Most mitigation options had a medium to large mitigating effect, but for some options, particularly those related to animal feeding and crop management, the range of effect included a potential increase in emissions. We concluded that sufficient evidence in the literature exists for a limited number of mitigation options, with a particular lack of LCA studies. There is a risk that greenhouse gas emissions could increase when implementing certain mitigation options, depending on current farming practices and implementation methods. The developed catalogue can help farmers and advisors to identify climate solutions for their farms and to avoid small or adverse effects by understanding the mechanisms of effects and possible causes of variation.

330 Supplementing *Gliricidia sepium* and coffee pulp to reduce methane production in tropical cattle of Mexico

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Due to climate change it is necessary to develop sustainable feeding strategies for cattle that do not compete with humans for feed resources. These strategies must help reduce enteric methane (**CH₄**) production; the main greenhouse gas produced by the agricultural sector. *Gliricidia sepium* (**GSep**) is a tropical legume tree native to Mexico that has showed potential to reduce CH₄ production. The objective of the present study was to evaluate the effect of GSep alone or associated with coffee pulp (**PCaf**) on enteric CH₄ production. Four treatments were evaluated Con=no additive, PCaf= 1.0 kg DM/d, GSep= 0.342 kg DM/d, and PCaf + GSep= 0.505 and 0.171 kg DM/d, respectively. Results from previous experiments conducted by our group on the effect of GSep and PCaf were used as indicative to determine the inclusion level of these additives for the present experiment. A Latin square experimental design was used, and the four treatments were randomly assigned to four crossed Charolais x Holstein heifers with an average live weight of 389 kg. The experimental diet consisted of 68.1 % maize straw, 14.8% ground corn, 4.4 % molasses, 8.7% bakery residues, 0.9% dry alfalfa meal, 2.2 % wheat bran, and 0.9% soya bean meal. The forage: concentrate ratio was 70:30, and diets were isoproteic and isoenergetic. The experiment lasted 96 days with four experimental periods of 21 days each, 19 days were for diet adaptation and three for measurements. Four days were used for as washout period between treatments. The CH₄ production was determined in two open-circuit respiration chambers for 48 h, and apparent digestibility was calculated by collecting total faeces production, which was subtracted (dry matter basis) from the daily dry matter intake (**DMI**). The daily CH₄ emission was 152.7, 144.4 and 140 g/d for PCaf, GSep and PCaf + GSep, respectively. These results indicate that treatments GSep and PCaf + GSep significantly reduced ($P<0.05$) enteric CH₄ production by 14.2% and 16.8%, respectively, compared with the Con treatment, 168.4 g/d. The highest antimethanogenic effect of treatment PCaf + GSep could be attributed to plant secondary metabolites in both PCaf and GSep, particularly condensed tannins. There were no significant differences ($P>0.05$) for DMI, 9.4, 9.2, 8.9 and 8.6 kg DM/d for Con, PCaf, GSep and PCaf + GSep, respectively, where the PCaf+GSep treatment showed consumption of 0.8 kg higher than the control but with a lower CH₄ production. The CH₄ yield (g CH₄/kg of DMI) showed no significant differences between treatments ($P>0.05$). The CH₄ yields are within the expected ranges for the type of diet administered, 17.9, 16.7, 16.3 and 16.5 g CH₄/kg DMI. Furthermore, decrease in daily CH₄ emissions was not at the expense of digestibility, particularly for the PCaf+GSep treatment compared with the Con, 47.8% and 47.9%, respectively. GSep and PCaf could be a natural and sustainable strategy to mitigate enteric CH₄ production in Mexico because GSep is already used as a live fence, and PCaf is an abundant agricultural by-product. So, farmers can easily implement this technology.

336 Carbon footprint of wean-finish pigs under different feeding management

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The increasing commercialisation of the Nigeria's pig industry to meet the projected demand for meat could lead to higher greenhouse gas emissions in the region, especially where the operations depend largely on concentrated feeds. To date, no country-wide study has estimated emissions across different growth phases of pigs fed various combinations of basal diets, and this study aims to fill that gap. This study estimated the carbon footprint (**CF**) in 117 weaned and 81 growing-finishing crossbred (Large White x Hampshire) pigs fed either of three basal-diets (maize, cassava plant meal 1 (**CPM 1**) or cassava plant meal 2 (**CPM 2**)) using the IPCC Tier 2 methods. The CPM 1 contained sun-dried unpeeled cassava tuber meal with sun-dried cassava leaf meal and sun-dried tender cassava stem meal mixed in ratio 2.5:1 while the CPM 2 contained the same cassava components but mixed at a higher ratio of 3:1. The maize-based diet contained crushed maize grain. All the CPM-basal diets were more digestible than maize-based diet. However, the CPM 2 diet is more digestible than CPM 1 due to more inclusion of highly digestible unpeeled cassava tuber meal. Performance data (daily weight gain, mortality, feed offered, feed refusal and feed intake), manure management systems and on-farm energy use were taken on weaner pigs (initial weight: 10±0.23 Kg) until they reached 20Kg. The growing-finishing stage lasted until pigs reached a live-weight of 90Kg at approximately 155 days. Results of CF estimated for the weaner pigs fed maize, CPM 1 and CPM 2-based diets were 23.95; 28.69 and 25.45 kgCO_{2e}/Kg live weight. In the growing-finishing phase which represents over two-thirds of the entire growth cycle, the CF were 49.44, 46.08 and 41.69 kgCO_{2e}/ Kg live weight for the pigs fed maize, CPM 1 and CPM 2-based diets respectively. The percent reduction was estimated as the difference between the CF arising from feeding the various combinations of CPM-basal diet as a proportion of the estimate obtained from maize-based diet. The results show a reduction of CF up to 7% and 15.5% for feeding CPM 1 and CPM 2 in diets of wean-finish pigs. The results suggest that, CF increases with increasing body weight of pigs and that cassava plant meal-based diets have the potentials for reducing the CF of Nigeria intensive pig production system.

339 Effect of yeast-treated wheat straw on milk production and methane emissions in crossbred dairy cows

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Addressing global food security and climate challenges requires innovative strategies that reduce waste while enhancing livestock productivity. When unutilized, liquid brewery yeast (**BSY**) poses environmental risks; however, livestock can convert it into valuable human food products such as milk and meat. BSY has high crude protein (**CP**) content and improves the digestibility of low-quality roughage. Moreover, improving roughage qualities reduces methane emissions from ruminants, a growing concern in countries like Ethiopia. This study aimed to evaluate the effects of BSY-treated/ensiled wheat straw (**EWS**) on feed intake, digestibility, milk and methane production, and economics compared to untreated wheat straw (**WS**), while exploring BSY as a moderate carbon footprint. BSY was applied at a rate of 0.6 liters per kilogram of WS, and molasses was added at 10% of the total weight of the mixture and fermented anaerobically in a small silo for five weeks. 200 mg samples were incubated with 1:5 rumen fluids to buffer solutions, respectively, for 24 hours. CH₄ yield was estimated using the formula (R²=88): CH₄ (MJ/d) = 0.3989 (±1.1073) + 0.8685 (±0.1585) × DMI + 0.6675 (±0.4264) × NDFI. Four mid-lactating high-grade (Friesian × Borena) dairy cows with an average body weight (400.19 ± 10.54 kg) and milk yield (8.01 ± 0.41 kg/d, mean ± SE) and parity of primiparous and multiparous were randomly assigned to one of four treatments using a 4 × 4 Latin square design, with each period lasting 25 days. The treatments were T1 = cows were fed WS ad libitum with 0.5 kg of concentrate per liter of milk (**CLM**); T2 = cows were fed EWS ad libitum with 0.3 kg of CLM; T3 = cows were fed EWS ad libitum with 0.5 kg of CLM; and T4 = cows were fed EWS ad libitum with 0.7 kg of CLM. EWS had significantly lower neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) contents, while CP and organic matter (**OM**) digestibility were significantly higher (p < 0.01) compared to UWS. In vitro total gases and CH₄ production in EWS were reduced (p < 0.01) by 18.1%, from 195 to 165 (ml⁻¹ g DM), and 28.4%, from 35 to 25.05 ml⁻¹g DM, respectively, compared to UWS. Parity was not affected by parameters. Dry matter, OM, CP, NDF, and ADF intakes and digestibility exhibited variation (p < 0.05), while NDF digestibility showed no variation. Milk yield and fat-corrected milk yield were significantly increased (p < 0.001) in T4 by 24% and 30%, respectively, compared to T1. Fat, protein, and lactose yields and milk production efficiency were significantly higher (p < 0.01) in T4 than in T1. CH₄ yield and CH₄ intensity were significantly reduced (p < 0.01) by 21.8% (from 35.8 to 28 g/kg DMI) and by 9.5% (from 46.8 to 30.5 g/kg of milk) between T1 and T4. Net profit was increased by \$1.21 per day per cow in T2 compared to T1. Financial benefits were maximized in T2, while a greater environmental benefit was observed in T4. EWS optimizes economic and environmental outcomes by utilizing agro-industrial by-products and enhancing dairy production.

341 The effect of sward type on enteric methane and rumen microbiome of ewes

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The rumen microbiome plays a fundamental role in the fermentation and digestion of feed, affecting animal performance, health and enteric methane production. Research in swards containing legumes and herbs has demonstrated improved animal performance in response to their inclusion. Yet, a paucity of information exists regarding the effect of sward species composition on enteric methane emissions of sheep and the associated rumen microbial community. This study compared the enteric methane output and rumen microbial profile of ewes, grazing a perennial ryegrass or a multispecies sward at two production stages.

Sixty ewes consumed a forage only diet, assigned to one of two forage types (n=30); A Perennial ryegrass (**PRG**; *Lolium perenne* L.) sward receiving 163kg N ha⁻¹ year⁻¹ or a five species multispecies sward (**MSS**) composed of perennial ryegrass, red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), chicory (*Cichorium intybus* L.) and ribwort plantain (*Plantago lanceolata* L.) receiving 90kg N ha⁻¹ year⁻¹. A single timepoint measurement was conducted on lactating ewes in May, and from the same ewes when dry (not lactating) in July 2023. Animals were fasted and weighed one hour prior to entering a Portable Accumulation Chamber (**PAC**) for 50 minutes to quantify enteric methane (O'Connor et al., 2021). Animals were then removed from the PAC and rumen fluid was collected using an oesophageal rumen sampler. Rumen microbial DNA was extracted and the V3-V4 region of 16S rRNA was sequenced using the Illumina MiSeq platform. Sequence data was processed using DADA2, and taxonomic classification was conducted using RefSeq + RDP database.

No treatment difference was recorded in daily methane output (38.0 vs 40.3 g CH₄ day⁻¹; P > 0.05) or methane intensity (0.51 vs 0.54 g CH₄ kg⁻¹ liveweight; P > 0.05) for lactating ewes. When dry, ewes grazing MSS had a lower enteric CH₄ output (14.9 vs 24.4g CH₄ day⁻¹; P < 0.001) and a lower enteric methane intensity (0.21 vs 0.35g CH₄ kg⁻¹ liveweight; P < 0.001) than those grazing PRG. Alpha diversity was higher in lactating ewes grazing MSS than those grazing the PRG sward (P < 0.05), while no treatment difference was observed in dry ewes (P > 0.05). The genera *Fibrobacter*, *Lachnospiraceae* FE2018 group, *Methanosphaera* and *Monoglobus* were more abundant in lactating ewes grazing MSS compared with those grazing PRG (P < 0.05). In the dry ewes grazing MSS, the genera *Methanosphaera* was higher while the genera including *Succiniclasicum*, *Selenomonas*, and *Anaerovibrio* were lower compared with ewes grazing PRG (P < 0.05). It is beyond the scope of the current study to compare responses between lactating and dry ewes.

Grazing MSS reduced the daily enteric methane output and intensity of ewes when dry, but not when lactating. Furthermore, grazing MSS altered the rumen microbial diversity in dry ewes and impacted microbial composition in both lactating and dry ewes. Further research is needed to evaluate these associations between microbial changes and enteric methane emissions.

342 Investigation of the effect of 3-nitrooxypropanol in dairy cow feeding

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The aim of this study is to examine the long-term effects of feeding 3-NOP on the methane excretion of dairy cows. The trial included one complete lactation and the early dry period. The data presented in this abstract cover the first seven month of the experiment, the data for the entire lactation are currently being analysed. The trial was conducted with thirty-two Simmental and sixteen Brown Swiss cows at Bayerische Staatsgüter, Research and Education Center for cattle Feeding Achselschwang. The animals were divided evenly into a control group (**CON**) and an experimental group with a 3-NOP supplement (**NOP**) from the 10th day of lactation on basis of lactation performance, breed and age. The cows received a maize- and grass silage-based TMR. The forage to concentrate ratio depended on lactation stage. In the NOP group, each cow received 185g of a mineral premix containing 0,8% 3-NOP per day, while in the CON group the corresponding amount of a placebo was added. The methane emission of the cows was measured using two GreenFeed stations (C-Lock Inc., Rapid City, South Dakota, USA). Furthermore, daily feed and nutrient intake was recorded via weighing troughs along with milk yield and composition, weight development, body condition score and backfat thickness. The statistical analysis was performed using the SAS statistical package (version 9.4, SAS Institute, Cary, NC, USA) using the 'MIXED' procedure and Bonferroni correction. In the model, the date, treatment, lactation number, lactation week and correction factors according to Ali and Schäffler were taken as fixed effects. Cow, clustered by group membership, was considered a random effect. The significance level was set at $p < 0.05$. 3-NOP did not affect total DM intake (24.1 kg/d for CON and 23.0 kg/d for NOP; $P = 0.119$). Since diet composition was identically for the feeding groups except for the addition of 3-NOP, the daily intake of energy, utilizable crude protein, starch and sugar did not differ between the experimental groups. 3-NOP did not affect daily milk yield (39.7 and 37.8 kg/d for CON and NOP; $P = 0.156$). The milk fat content was 4.02% in the NOP group and 3.82% in the CON group ($P = 0.07$). The milk protein content (CON: 3.53; NOP: 3.60%; $P = 0.160$) and the ECM yield (CON: 39.3 kg/day; NOP: 38.5 kg/day; $P = 0.525$) did not differ between the groups. Daily methane emission was 291 g/cow for NOP and 421 g/cow for control ($P < 0.001$). Methane intensity was 11.0 and 7.84 g/kg ECM for CON and NOP ($P < 0.001$). Methane yield was 18.3 and 13.2 g/kg DM for CON and NOP ($P < 0.001$). This corresponds to a reduction of methane emission of 31 – 28 % in NOP compared to CON. There are some indications that as cows get closer to the dry period and eat less, the differences between groups became lower. Potential relationships with diet composition, nutrient content and days in milk are currently being investigated.

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348 Enteric methane emissions from beef cattle grazing silvopastoral vs. open-sky pastures in northeastern Argentina.

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Silvopastoral systems are a promising strategy to improve the carbon balance in beef cattle production. These systems integrate forage production with timber trees within the same space and time, promoting ecological complementarity among components. Such interactions can enhance biomass productivity and, consequently, increase carbon inputs. In addition to their environmental benefits, silvopastoral systems may also improve animal welfare and pasture nutritional quality. This ongoing study aims to compare enteric methane (**CH₄**) emissions between two grazing systems: open-sky (**OS**) and silvopastoral (**SP**). A long-term experiment was established to characterize CH₄ emissions over three observation periods: August and November 2024, and March 2025. The OS system comprises a 3.5-hectare pasture dominated by *Urochloa brizantha* cv. Marandú, while the SP system features a 3.5-hectare pasture of *Axonopus catarinensis* Vaals under a canopy of 143 *Pinus elliottii* trees per hectare. These forage species are representative of their respective systems in the study region. At the beginning of the experiment, twenty Brangus steers were allocated to each treatment, balanced by initial live weight (**LW**), which averaged 219 ± 24 kg in the SP group and 213 ± 18 kg in the OS group. Both groups were managed under a rotational grazing strategy with 7-day paddock occupancy. Forage allowance was standardized at 3000 kg DM per treatment at the beginning of each period. Pasture samples were collected from each system to determine dry matter (**DM**), crude protein (**CP**), neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**) contents. LW was recorded every 21 days to adjust paddock sizes accordingly. During each observation period, individual dry matter intake (**DMI**) was estimated using titanium dioxide (**TiO₂**) as an external marker, while indigestible NDF was used to estimate individual DM digestibility. Enteric CH₄ emissions were measured over five consecutive days using the SF₆ tracer technique. Preliminary results from the first observation period showed no significant differences between systems in average daily gain (**ADG**) ($P = 0.41$; 0.36 kg/d in SP and 0.32 kg/d in OS), CH₄ emissions ($P = 0.15$; 147.1 g/d in SP and 129.1 g/d in OS), or CH₄ intensity ($P = 0.82$; 437.04 and 458.4 g CH₄/kg ADG, respectively). However, analyses of forage chemical composition and DMI are still underway. Completion of these assessments is essential to gain a more comprehensive understanding of the productivity and environmental performance of these contrasting systems. Final results will enable a better evaluation of how seasonal changes in pasture composition and system design could influence CH₄ emissions in growing beef cattle. Full findings will be presented during the conference.

349 Impact of calcium peroxide on enteric methane emissions in grazing lactating dairy cows

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Mitigating methane (**CH₄**) emissions in grazing systems remains challenging due to limited efficacy of anti-methanogenic additives. Previous studies demonstrated up to a 30% reduction in CH₄ emissions using a calcium peroxide (CaO₂) additive in indoor beef systems without impacting performance. This study aimed to evaluate the impact of CaO₂, supplemented twice daily via a concentrate feed, on milk production and enteric CH₄ emissions in grazing lactating Holstein Friesian dairy cows.

Forty multiparous spring-calving Holstein Friesian dairy cows were blocked by parity and days in milk (52 ± 11.1) and balanced for previous 305-d milk solids yield (556 ± 91.6 kg), pre-experimental body condition score (2.8 ± 0.24), body weight (562 ± 47.1 kg) and CH₄ emissions (266 ± 44.3 g d⁻¹). Cows were randomly allocated to one of two dietary concentrate treatments containing 7 kg d⁻¹ of concentrate; either 0 g kg⁻¹ DM of CaO₂ (Control; **CTR**) or 45 g kg⁻¹ DM of CaO₂ (**CaO₂**). Cows grazed as one group and were allocated 14 kg DM d⁻¹ of fresh pasture. The 9-week experiment included a 14-d dietary acclimatization period, followed by a 49-d CH₄ measurement period using GreenFeed (**GEM**). A pasture intake assessment period occurred during weeks 3 and 4 using the n-alkane method. Rumen esophageal sampling was conducted on day 26 and 63 to determine volatile fatty acid (**VFA**) concentration. Data were analyzed using the MIXED procedure of SAS® studio. The model for gaseous emissions included the fixed effects of treatment and parity, with days in milk and pre-experimental body weight as covariates. For milk production parameters, the model included the fixed effects of treatment, week, parity and treatment by week interaction, with days in milk and pre-experimental milk solids as covariates. The model for rumen fermentation parameters included the fixed effects of treatment, day, parity and treatment by day interaction, with days in milk included as a covariate.

The supplementation of CaO₂ did not affect milk yield (CTR: 35.4 kg d⁻¹, CaO₂: 36.2 kg d⁻¹), milk solids yield (CTR: 2.74 kg d⁻¹, CaO₂: 2.79 kg d⁻¹) or milk composition ($P > 0.05$). CaO₂ cows visited the GEM system more frequently (2.6 vs 2.0 visits/d; $P < 0.05$) and consumed more GEM concentrate (0.63 vs 0.48 kg d⁻¹; $P < 0.05$). Absolute CH₄ emissions were higher in CaO₂ cows (353.8 vs 321.4 g d⁻¹; $P < 0.05$), but CH₄ intensity (g/kg milk solids) was unaffected ($P > 0.05$). Hydrogen (H₂) emissions were reduced in CaO₂ cows (1.58 vs 1.92 g d⁻¹; $P < 0.05$). There was no effect of treatment on ruminal total VFA concentration ($P > 0.05$), however, the acetate to propionate ratio was greater for CaO₂ cows (3.71 vs 3.05; $P < 0.05$).

Calcium peroxide increased CH₄ emissions by 9% but reduced H₂ emissions by 17% without affecting milk production or composition. Further research into the effects of CaO₂ and its potential role in grazing dairy systems is required.

350 Effects of persistent and pulse dosing calcium peroxide on physiochemical and microbes in dual-flow fermenters

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Methane from ruminants is a significant contributor to greenhouse gases, with microbial methanogenesis in the rumen being the key site. Calcium peroxide, an oxidizer, alters the oxidation-reduction potential (**ORP**) of the rumen offering a potential strategy for mitigating methane as methanogens are sensitive to positive ORP fluctuations. This study investigates persistent and pulse dosing of calcium peroxide to sustain elevated ORP and inhibit methanogenesis while maintaining fermentation stability.

Three Dual-Flow Fermentor (**DFF**) systems were used, and each experiment was repeated in triplicate. Each fermenter contained 800mL of rumen fluid sources from fistulate cows fed a 50:50 forage-to-concentrate diet. The experiment consisted of a 7-day adaptation phase followed by a 5-day analysis period, with three experimental groups:

- 1) *Control Group*: Standard conditions with no calcium peroxide addition.
- 2) *Pulse Dose Group*: Received calcium peroxide twice daily in shock doses
- 3) *Persistent Dose Group*: Received the same total daily dose as the Pulse Group, administered periodically throughout 24 hours.

Fermenters were maintained at 39°C with continuous agitation. Artificial saliva infusion (122mL/hour), along with liquid and solid outflow rates were kept consistent to mimic rumen function and passage. Each fermenter was fed 20g of dry matter daily, 10g in the morning and evening at the same time daily. Methane production, ORP, volatile fatty acid (**VFA**) profiles and pH were measured to assess fermentation dynamics and the impact of calcium peroxide dosing strategies.

Drawing from trends reported in recent investigations (O'Donnell *et al.*, 2024; Roskam *et al.*, 2024; Graham *et al.*, 2024), we anticipate that pulse dosing of calcium peroxide may cause elevations on ORP for short periods of time. These short increases might temporarily cause a methane reduction but would likely allow emissions to rise again prior to subsequent doses. In contrast, we expect a more sustained (persistent) dosing regimen to maintain elevated ORP over the 24-hour period causing a prolonged methane reduction. Based on these findings, pH and VFA profiles are not altered significantly, suggesting that fermentation stability would remain unaffected by both dosing strategies.

If our results align with those observed in (O'Donnell *et al.*, 2024; Roskam *et al.*, 2024; Graham *et al.*, 2024), we would conclude that the persistent method is more effective than the pulse method in consistent reduction of methane production. Maintaining elevated ORP appears to be very important in achieving a sustained methane suppression without negatively impacting fermentation stability. Future research, including long-term *in vitro* and *in vivo* studies will be required for refining dosing approaches and fully understanding their implications for rumen health and animal performance.

352 A combination of by-products significantly reduces the enteric methane emissions from sheep in Argentina

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Livestock contributes to climate change but is vital for meeting the growing global demand for animal protein. The inclusion of by-products into ruminant diets can improve food circularity within agriculture and reduce the competition for human-edible resources. In addition, the inclusion by-products in ruminant diets represents a potential trade-off as some of them have the potential of decreasing enteric methane (**CH₄**) emissions. This study aimed to evaluate the effect of replacing maize grain and urea (control diet, **CON**) with locally available by-products on sheep CH₄ emissions, either by using maize-based distiller grains (**DDGS**) combined with barley brewed grains (**BAR**) or wheat middlings (**WHE**). Diets were formulated to be isoproteic and isoenergetic (2.6 Mcal ME kg DM⁻¹ and 160 g CP kg DM⁻¹) with a corn silage-to-concentrate ratio of 0.60:0.40 (on a dry matter basis). Twelve Highlander ewes, around one year old, with an average initial live weight (**LW**) of 35.9 ± 3.12 kg (mean ± SD; n=4 per treatment) were used in a Completely Randomized Block design with two periods, four blocks, and three animals per block. Individual animals were the experimental unit. The statistical analysis was performed with the function lme from nlme package of R (version 4.3.3). The study lasted for 60 days, consisting of a 30-day acclimatization to diets and chambers, and 30 days of measurements. Ewes were housed individually in respiration chambers for 48 h for measuring entericCH₄ emissions. Individual LW was recorded weekly and used to adjust diet allocation fortnightly. Dry matter intake was restricted at 2.5% of LW. Overall, daily CH₄ production was not affected by dietary treatment and averaged 9.64 ± 0.594 g d⁻¹ (mean ± SEM). However, the BAR diet reduced CH₄ emitted per kg of LW by 25% ($P<0.01$), and CH₄ yield by 28% ($P<0.01$) when compared to the CON diet, respectively. In conclusion, DDGS when offered with barley brewed grains or wheat middlings can effectively replace corn grain and urea in sheep diets without increasing total CH₄ production. Both CH₄ intensity and yield were significantly reduced when maize grain and urea are replaced by DDGs and barley brewed grains.

354 Evaluating feed additives to mitigate ruminants' methane emissions: In vitro insights using sheep ruminal fluid.

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With global populations set to rise to an estimated 10 billion by 2050 it is critical that food production not only meets this demand but does so in an efficient and environmentally friendly manner. Ruminants are crucial to global food security, providing a vast array of meats, milk and dairy products. However, they are responsible for 90% of the methane produced in the agriculture industry. One approach to addressing this involves reducing the amount of enteric methane produced naturally by ruminants via the use of feed additives. With that in mind, this study aimed to evaluate the effectiveness of nine natural feed additives in reducing methane emissions *in vitro*, using sheep ruminal fluid, whilst monitoring their impacts on other key fermentation characteristics.

Nine natural additives were tested including four variations of *Solieria chordalis*, three *Ulva* and two test products for Olmix, Feed additive 1 and 2. These were evaluated against a control of grass silage (*Lolium perenne*), a positive control (*Asparagopsis taxiformis*), and a blank, containing no substrate. Experiments were carried out across three separate *in vitro* fermentation runs using sheep ruminal fluid collected at slaughter. In each run, ruminal fluid from five unique animals was collected and equally homogenised, strained and mixed with Van Soest buffer at a 1:1 ratio. Each additive was evaluated in triplicate per *in vitro* and added at 80 mg/g of the basal diet, with the exception of Feed additive 1, which was included at 22.5 mg/g. A total of 60 ml of the ruminal fluid-Van Soest mix was added to a combined 1.6 g of substrate per jar. The samples were then fermented anaerobically at 39°C and 95 rpm for 24 hours prior to analysis. Analysis included gas pressure, pH, methane production, ammonia concentration and digestibility. Data were statistically examined in R using analysis of variance and Tukey tests.

An analysis of variance was carried out which determined a significant difference in methane production between the sample groups, $F(11, 31) = 27.05$, $p < 0.001$. A post-hoc Tukey test highlighted a significant difference between Feed additive 1 and the control ($p < 0.01$). A 41.25% reduction in methane was observed when compared to the control, with the mean methane produced decreasing from 3.82 ± 0.03 ml to 2.24 ± 0.03 ml after 24 hours (mean $\pm$ SEM). Additionally, total gas production was reduced by 14.63%, and digestibility decreased by 10.69%, whereas ammonia production was increased by 15.95% compared to the control. However, all changes were non-significant. No additional additives had a significant effect on methane production, nor did any additives present a significant change in total gas production, ammonia production or digestibility when compared to the control.

Out of the nine additives tested, Feed additive 1 was the only one that significantly reduced methane production compared to the control. This compound shows promising potential to reduce methane production however further research, focusing on lower inclusion levels and *in vivo* trials, is essential to assess its feasibility for practical application in the livestock sector.

355 Methane reduction in cattle diets through tropical legume pods and foliage supplementation

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Methane (**CH₄**) emissions from ruminants are a major contributor to greenhouse gases, particularly in tropical regions where cattle diets are often low in quality. This study integrated findings from two experiments to evaluate the potential of tropical legume pods and foliage, specifically *Samanea saman*, *Enterolobium cyclocarpum* with *Gliricidia sepium*, to mitigate CH₄ emissions, enhance nutrient digestibility, and modify rumen fermentation patterns in cattle.

The experiment was conducted using crossbred heifers (*Bos indicus* × *Bos taurus*) under controlled conditions. Control diets consisted of tropical grass (*Pennisetum purpureum* or *Brachiaria brizantha*) supplemented with varying proportions of legume pods and foliage. The experimental designs were replicated Latin squares with CH₄ emissions measured using open-circuit respiration chambers. Key variables included dry matter intake (**DMI**), apparent nutrient digestibility, and CH₄ production expressed as liters per day or per kilogram of dry matter intake (L CH₄/kg DMI). Rumen fermentation parameters, such as volatile fatty acid (**VFA**) proportions and acetic:propionic acid ratios, were assessed using chromatography.

Inclusion of *S. saman* pods reduced CH₄ emissions by up to 50.9% compared to control diets. A significant linear reduction in CH₄ output was observed with increasing pod inclusion levels. Diets incorporating pods of *E. cyclocarpum* and foliage of *G. sepium* showed a CH₄ reduction of 10-21% per unit of dry matter degraded compared to control. *S. saman* supplementation did not affect overall DMI or apparent digestibility of dry matter (591–635 g/kg intake). *E. cyclocarpum* and *G. sepium* reduced fiber digestibility due to tannins but increased crude protein (**CP**) digestibility by 14% on average. Diets supplemented with *S. saman* pods significantly increased propionic acid molar proportions and decreased the acetic:propionic acid ratio, indicating a shift towards more efficient fermentation. *E. cyclocarpum* and *G. sepium* inclusion showed a linear increase in butyric acid proportions while maintaining stable pH levels.

Supplementing cattle diets with tropical legume pods and foliage can significantly mitigate enteric CH₄ emissions without negatively impacting feed intake or overall productivity. *S. saman* pods were particularly effective in reducing CH₄ emissions, while *E. cyclocarpum* and *G. sepium* balanced CH₄ mitigation with improved protein digestibility. These findings highlight the potential of strategic tropical legume supplementation to enhance the sustainability of tropical livestock systems. Future studies should explore seasonal variations and optimize supplementation levels for practical application.

357 Effect of agro-industrial by-products supplementation on cow productivity and methane emission in the Peruvian Amazon

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Livestock farming in the Peruvian Amazon faces the challenge of increasing productivity while minimizing environmental impacts, especially enteric methane emissions. Livestock feeding, based mainly on low-quality pastures, contributes to these emissions and limits productivity. A promising strategy to address this problem is feed supplementation with local agro-industrial by-products, which are often discarded. The use of these by-products would increase nutrient circularity and the resilience of livestock systems.

The experiment was carried out in the San Martín region (Peru). Twelve cows were randomly assigned to three groups, each receiving a different diet. Control diet (diet A) consisted of commonly used regional inputs, including ground corn, wheat bran and soybean cake. Diet B incorporated cocoa husk at 30% of concentrate offered, while diet C included rice bran at 30% of concentrate offered. The animals grazed *Urochloa brizantha* all the time and were supplemented with the experimental concentrates (4 kg per day) during milking. The experiment was carried out following a 3 x 3 Latin square design, using diets and stages as rows and columns. The estimation of organic matter intake per animal was done using titanium dioxide marker. Milk produced per animal in the morning and afternoon milking was weighed individually. These measures were carried out for 6 continuous days. The concentration of fat, non-fat solids protein, lactose, total solids, density and urea in milk will be carried out with the MILKOSCAN FT1 equipment. Feed samples were analysed for dry matter, protein and ether extract according to the methodology proposed by the Association of Analytical Chemists, neutral detergent fiber according to the Van Soest method and in vitro digestibility of dry matter for 48 hours was determined with the Ankom DaisyII Incubat® incubator. Enteric methane emissions will be estimated using recent dairy cattle-specific prediction equations based on productive traits, dietary traits, and feed intake.

The cows showed acceptance and voluntary consumption of the agro-industrial by-products in the diets, with no rejection or decrease in total dry matter intake. Analysis of quantitative data from the comparison of diets is ongoing, but it is expected that the use of these by-products will not decrease milk production compared to the control diet and will contribute to the reduction of enteric methane emission. Within the framework of a circular economy, this study seeks to contribute to the exploration of lesser-known feed resources, contributing to waste minimization and sustainability of the dairy production systems in the Amazon region.

359 Metabolomic profiling of forages to identify anti-methanogenic compounds targeting enteric methane for sustainable livestock systems

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Ruminant methane (**CH₄**) emissions are a major contributor to global greenhouse gas levels, highlighting the urgent need for sustainable mitigation strategies. This study, conducted as part of the Anti-Methanogenic Feedstock for Livestock Systems in the Global South project led by CIAT, aims to identify anti-methanogenic compounds (**AMCs**) in forages using an integrative metabolomics approach combined with in vitro CH₄ production assays. Identifying AMCs in livestock feed can support CH₄ mitigation – through selection or genetic optimization – while maintaining animal productivity. We employ advanced normalized untargeted metabolomics, specifically **LC/MS**-based techniques (liquid chromatography coupled with mass spectrometry), in conjunction with in vitro emissions data, to identify molecular targets with methane-reducing potential. To pinpoint relevant AMCs, we apply statistical analyses, unsupervised classification, and differential analysis of metabolomic profiles from contrasting high- and low-CH₄-producing samples. This enables the identification of under- or over-expressed metabolites (by mass) associated with methanogenic activity. A critical step in this process is the structural and functional annotation of individual compounds, inferred from LC/MS mass spectra. However, only a small fraction (~5–6%) of the compounds in these forage samples have been structurally resolved and annotated in public databases. To address this bottleneck, we have developed a machine-learning-based pipeline to predict, validate, and identify previously uncharacterized metabolites. This involves: predicting potential molecular structures that match the experimental mass spectra using a trained neural network; generating in silico single-electron impact MS for each predicted structure; and comparing the predicted spectra with the experimental data using dot-product similarity scoring to confirm the best structural matches. We are currently testing and refining this tool to improve the fraction of fully resolved compounds in our forage metabolite database. Progress in the development and application of this pipeline will be reported during this session. Preliminary results of three forage species – *Leucaena diversifolia*, *Cratylia argentea*, and *Urochloa humidicola* – revealed different flavonoids and saponins previously reported in the literature as AMCs. Notably, *Leucaena diversifolia* exhibited high flavonoid levels, which correlated with reduced in vitro CH₄ production, positioning it as a promising candidate for further study. Additionally, across these three species, we found that lyophilization significantly enhances the preservation of volatile and thermolabile metabolites compared to heat-drying, as evidenced by higher peak intensities in the mass spectra ($p < 0.05$). To broaden the search space across plant families, species, phenotypes, and potential AMCs, we have obtained LC/MS data from 26 samples representing 13 forage species, collected from three CGIAR centers: ICARDA, ILRI, and CIAT. Within the species *Trifolium*, *Leucaena*, *Erythrina*, *Panicum*, *Stylosanthes*, *Lathyrus*, *Vicia*, *Desmodium*, *Chloris*, *Cenchrus*, *Flemingia*, *Cajanus*, and *Hedysarum*, we selected sample pairs with contrasting CH₄ production profiles. We will present during this session a list of metabolites identified in these forages, associated with either positive or negative methanogenic activity. We expect this comprehensive approach will guide the development of forage-based feeding strategies and gene-edited forage varieties, ultimately contributing to sustainable livestock systems and addressing the dual challenges of climate change and food security in the Global South and beyond.

362 Calcium peroxide supplementation mitigates methane with no effects on animal performance in grazing beef cattle

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Dietary supplementation with methane mitigating compounds has been recommended as an effective anti-methanogenic strategy, however, only a few compounds to-date have proven effective in pasture-based production systems. Calcium peroxide (**CaO₂**) has been reported to reduce methane production *in vitro* and in housed beef cattle, through altering the redox potential of the rumen. This study investigates the effects of offering CaO₂ mixed into concentrate feed to grazing beef cattle on gaseous emissions and animal performance.

Fifty Charolais X animals with an average bodyweight of 501 ± 38 kg and age of 15.5 ± 0.6 months were assigned to a 10 wk study. Animals were blocked by bodyweight and randomly assigned within block to two grazing groups; **TRT** (n=25) and **CON** (n=25), with each treatment grazed in separate paddocks. Animals were allocated fresh pasture every 24 h. Each animal was offered 0.75 kg concentrates every morning and evening, CON received concentrates with 0% CaO₂, whereas TRT received concentrates containing 10% CaO₂ (targeting 2% inclusion of CaO₂ of the animal's total dry matter intake). Each group had access to a GreenFeed (C-Lock; Rapid City, South Dakota, USA) which was rotated between groups twice weekly to eliminate machine bias. Each animal received up to 1 kg additional concentrates per day from GreenFeed visitation. Animals were weighed fortnightly to facilitate calculation of average daily gain. Grass dry matter intake was estimated using the n-alkane technique. Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA). The statistical model included treatment, sex and dam breed as fixed effects, with animal included as a random effect.

Mean pre-grazing herbage mass was 1,817 and 2,024 kg DM/ha, while mean post grazing sward height was 4.86 and 4.71 cm, for CON and TRT, respectively. Both CON and TRT started with a similar bodyweight ($P = 0.19$). After the 10 wk supplementation period, average bodyweight remained similar for both groups ($P = 0.12$) with an average daily gain of 0.44 and 0.40 kg/d for CON and TRT, respectively ($P = 0.88$). Regarding estimated dry matter intake, TRT and CON consumed 7.10 and 8.43 kg DM/d, respectively ($P < 0.001$), however, this did not have an effect on gain:feed ratio ($P = 0.79$). Gaseous emissions (g/d), based on GreenFeed measurements over the 10-week supplementation period, resulted in TRT emitting less methane, hydrogen and carbon dioxide ($P < 0.001$) than CON, equating to reductions of 19, 52 and 11% respectively. Similarly, methane per kg bodyweight was lower for TRT than CON ($P < 0.001$). When expressing methane per unit of dry matter intake, there was no difference between CON and TRT ($P = 0.44$). Offering concentrates enriched with CaO₂ to grazing beef animals significantly reduced the production of enteric methane whilst maintaining a similar growth rate compared to their unsupplemented counterparts. As CaO₂ supplementation had a negative effect on dry matter intake, further investigation is required to ensure no negative effects on animal performance, health or welfare in longer-term studies.

364 Breeding *Urochloa* spp. and *Megathyrsus maximus* for low methane livestock systems

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Ruminant livestock is the primary global source of meat and milk, efficiently transforming vegetative material into human-edible protein. Agricultural grasslands cover two-thirds of the Earth's agricultural land, dominated in the tropics by forages of the genera *Urochloa* and *Megathyrsus* supporting over 800 million animals. Livestock plays a vital economic and social role. However, inadequate feed quality and management negatively impact both environmental sustainability and livestock productivity. This study aims to develop low-methane forages (**LMF**) to mitigate greenhouse gas emissions from livestock by introducing traits that will reduce enteric methane production into widely used forage-grass cultivars through conventional breeding methods. The LMF strategy focuses on highly digestible forages that minimize methane production per unit of dry matter digested, incorporating potentially anti-methanogenic compounds. The Alliance of Bioversity International and CIAT is evaluating elite genotypes and commercial cultivars from three breeding programs: Interspecific *Urochloa*, *Megathyrsus maximus* and *Urochloa humidicola* selected for agronomic performance and seed yield potential as a measure of scalability and environmental adaptation. In 2024, three separate field trials were established, one for each species of interest, in an alpha lattice with three reps and 5 plants per rep. These lines are being assessed for key agronomic traits such as forage production and nutritional quality including acid detergent fiber (**ADF**), neutral detergent fiber (**NDF**), truly digested dry matter (**TDDM**) and in vitro methane production (ml/g DM). The methane production and nutritional quality characterization started in 2025 and will continue until 2026. In 2025, three replicates per genotype (10 interspecific *Urochloa* genotypes, 10 *Megathyrsus maximus*, and 10 *Urochloa humidicola*) were sampled at the recommended height for each species. For the in vitro determination of methane production using the syringe methodology each sample was repeated three times resulting in a total of 9 replicates per genotype. Additionally, for each genotype tested, two sub-samples were freeze-dried and stored for future genomics and metabolomics studies. Preliminary results in terms of methane production indicated that the interspecific *Urochloa* genotypes sampled had an average 25.9 ± 6.7 CH₄ ml/g DM incubated for 24 h, and $64.1\% \pm 2.1$ TDDM. Regarding nutritional quality, fiber traits obtained showed 57.8% NDF and 23.3% ADF on average. The *U. humidicola* genotypes sampled had an average 36.0 ± 3.3 CH₄ ml/g DM, $57.2\% \pm 2.2$ TDDM, and an average of 68.4% NDF and 29.1% ADF. The *Megathyrsus maximus* genotypes sampled had an average 30.2 ± 4.6 CH₄ ml/g DM, $57.9\% \pm 3.7$ TDDM, and an average of 65.3% NDF and 29.8% ADF. There are significant differences between genotypes on each genus (Tukey $p < 0.05$). Once the remaining genotypes are characterized, the best genotypes will be selected using a decision matrix prioritizing low CH₄ production, high TDDM, nutritional quality parameters, agronomic performance and scalability.

367 Effects of whey and 3-nitroxypropanol on lactational performance and enteric methane emissions in dairy cows

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Nutritional strategies are important for enteric methane (CH_4) mitigations of dairy cows. Whey, a lactose-rich byproduct, can support the growth of lactic acid bacteria such as *Sharpea* and *Oribacterium*, which are associated with low CH_4 phenotypes in ruminants. Lactose fermentation produces lactate, which can be converted to propionate, reducing hydrogen availability and CH_4 emissions. In a recent *in vitro* study, synergistic CH_4 mitigation effect was observed between dietary supplementation of whey and 3-nitrooxypropanol (**3-NOP**; 48 vs. 28% for the combination and 3-NOP, respectively). The objective of this study was to determine the effects of whey and 3-NOP supplementation on lactational performance and CH_4 emissions in dairy cows. Twenty-four Holstein cows (177 ± 46 days in milk) were enrolled in a randomized complete block design experiment with a 2×2 factorial arrangements in a free-stall research barn. Cows were blocked by pre-treatment milk yield (7-day average) and randomly assigned to receive a basal (**BAS**; $n = 6$) or whey-mixed diet (**WHM**; 15% of DM; $n = 6$; Emmi AG, 6005 Lucerne, Switzerland), supplemented with either a placebo (**PCB**; $n = 6$) or 3-NOP ($n = 6$; Bovaer, 80 mg/kg TMR DM). The experiment lasted 13 weeks, including a 1-week covariate period when all cows were fed the BAS diet, followed by a 12-week experimental period. Treatment diets were balanced for crude protein, starch, and neutral detergent fiber contents (15.4%, 18.3%, and 38.3% of TMR DM, respectively). Cows were fed and milked $2 \times /d$. Individual feed intake was measured using a Calen-gate system. Enteric CH_4 emissions were measured using GreenFeed System. Data were analyzed using mixed models with the *lmer* procedure in R, including fixed effects of baseline measurement (covariate), whey, inhibitor, week, and up to three-way interactions among whey, inhibitor, and week, and random effects of block and cow within treatment. A tendency ($P = 0.10$) for the interaction between whey and inhibitor was observed for DMI, with cows fed WHM and supplemented with 3-NOP showing a slight decrease in DMI (BAS-PCB = 27.3; BAS-3-NOP = 26.0; WHM-PCB = 25.0; WHM-3-NOP = 25.2 kg/d). However, milk yield did not differ across treatments. Interactions between whey and inhibitor were not observed for enteric CH_4 emissions. Methane production was reduced in cows fed the WHM diet ($P = 0.05$) and those supplemented with 3-NOP ($P < 0.0001$) [(BAS-PCB = 476; BAS-3-NOP = 281; WHM-PCB = 423; WHM-3-NOP = 271 g/d)]. Although WHM-based diets did not affect CH_4 yield or intensity, 3-NOP supplementation reduced both (CH_4 yield: $P < 0.0001$; BAS-PCB = 17.0; BAS-3-NOP = 11.4; WHM-PCB = 17.0; WHM-3-NOP = 10.9 g/kg DMI; CH_4 intensity: $P < 0.0001$; BAS-PCB = 14.9; BAS-3-NOP = 8.36; WHM-PCB = 13.5; WHM-3-NOP = 8.57 g/d $\pm$ 0.65 g/kg of milk). In conclusion, no synergies were observed between 3-NOP and whey in mitigating enteric CH_4 emissions in dairy cows. Supplementing whey in dairy cow diet only reduced absolute CH_4 production, likely caused by reduced intake, and did not improve performance or reduce methane yield or intensity.

368 In vitro screening for low methane forages and their in vivo evaluation: approaches and pathways

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Reducing methane (**CH₄**) emissions from livestock systems is critical for mitigating climate change, especially in the Global South, where enteric CH₄ accounts for a significant proportion of livestock-related emissions. To address this challenge, three CGIAR Centres (listed in the authors' affiliations) have initiated a project to screen ~10% of 71,000 forage germplasm accessions, focusing on legumes and grasses, to identify low CH₄ forages (**LMFs**). LMFs are defined as highly digestible forages that produce low levels of CH₄ per unit of digested dry matter and may contain anti-methanogenic compounds (**AMCs**) such as tannins (polyphenols), saponins, hydrogen sinks, and inhibitors, among others. Cosmopolitan forage species will be targeted. The forages should consistently reduce CH₄ emissions by at least 12%, should be drought-tolerant, scalable, easy to cultivate, economically viable and capable of enhancing livestock productivity while reducing environmental and ecological impacts. Guided by this definition, a stepwise screening approach based on a decision matrix was developed to transition promising forages from in vitro evaluation to in vivo validation. The evaluation of LMFs follows a systematic three-phase framework: Phase 1: in vitro gas production analysis using the syringe method evaluates forage accessions based on three key indicators: truly digested dry matter (**TDDM**, %), CH₄ production (CH₄, % in gas), and CH₄ intensity (CH₄/unit of TDDM, ml/g). A decision matrix prioritizes forages with high TDDM, low CH₄, and low CH₄ intensity for advancement to Phase 2. Phase 2: Metabolic profiling, including tannin and saponin bioassays, confirms the presence of AMC. Thresholds for high, medium, and low levels of CH₄ emission in the matrix are being defined based on a pilot study with 150 forage species and cultivars from gene banks and supported by existing literature. These thresholds are expected to vary among legumes and grasses as well as between tropical and temperate forages and will be finalized based on the evidence gathered in phase 1. Phase 3: Selected forages will undergo in vivo validation using respiration chambers to measure CH₄ emissions under real feeding and environmental conditions. The decision matrix continues to guide the process, prioritizing forages with optimal CH₄ mitigation properties and identifying supplementation strategies. Ongoing activities will finalize Phases 1 and 2, and key results including decision matrix thresholds will be shared at the conference. Two approaches for implementation of LMFs in the production systems, termed as evolutionary and revolutionary area proposed. Evolutionary approach: Refines existing diets with locally available LMFs, targeting about 15% increase in productivity and 15-20% decrease in CH₄ emission. Revolutionary approach: Introduces novel or gene-edited LMFs, targeting >25% increases in both productivity and CH₄ emission reduction, but requiring greater adaptation and introduction efforts. All scaling options should consider ease of propagation (seed or vegetative), adaptability to local climate and soils, and potential for high yields, to ensure successful field implementation. It represents a critical step towards developing climate-smart livestock systems in the Global South where forage-based systems dominate livestock production and make forages to be the key delivery mechanism of enteric CH₄-reducing compounds to ruminants.

369 Effects of silage additives, fermentation products and feed preservatives on *in vitro* rumen fermentation products

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Cattle on grazed pasture produce less enteric methane (**CH₄**) than cattle fed grass silage indoors. Grass silage is prepared with different inoculants and/or silage additives (**SAd** e.g., Formic acid-based additive) to fresh or wilted material before packing for controlled fermentation. Furthermore, a well-preserved silage contains fermentation products (**SFp**) such as organic acids (e.g., propionic, acetic, lactic) among others. Lastly, once silage is ready for use, feed preservatives (**FPr**) are added to the silage or total mixed ration to improve its aerobic stability. The effects of these fermentation products and added organic acids on short chain volatile fatty acids (**VFA**) and CH₄ production are of practical importance. The objective of this study was to test the effects of SAd, SFp and FPr when added to fresh dried grass (**FG**) on total gas (**TG**), VFA and CH₄ production in the presence of grass silage (**GS**).

In vitro rumen fermentation was carried out using ANKOM RF gas production with FG and GS prepared from the same cut to test the effects of SFp (added as Formic acid 10 g/kg DM + Lactic acid, 40 g/kg DM), SAd (GrasAAT Plus, 6 L/ton wet feed) and FPr (GrasAAT TMR; 6 L/ton wet feed) on fermentation parameters. The SFp, SAd and FPr were added to FG creating the following dietary treatments: (1)- FG, (2)- FG+SFp, (3)- FG+SAd, (4)- FG+FPr, (5)- FG+SFp+FPr, and (6)- GS. For SFp and SAd, the quantities included were based on what was analysed in the GS, whereas for FPr, the amount equated to practical inclusion level for silage mixing to improve aerobic stability. The experiment was run in two batches of 24h duration containing triplicates. Data were analysed using SAS proc mixed (SAS, 2023) with treatments and batch as fixed effects and replicates as random. With significant treatment effects, multiple mean comparison was made with the GS treatment as a control.

TGP ($\pm$ SD) (225.5 ± 20.7 mL/g DM feed) and total VFA (89.9 ± 5.75 mMol/L) did not differ among the dietary treatments ($P > 0.10$). However, molar percentages of acetate decreased and that of propionate increased ($P < 0.05$) with the inclusion of FPr alone and FPr+SFp. On the other hand, the addition of SFp, SAd and FPr increased (over 10%; $P = 0.032$) CH₄ production (mL/g DM). The FG and GS treatments did not differ in CH₄ production and in the main VFAs. However, molar percentages of minor VFAs (i.e., valerate, isovalerate, and isobutyrate), and ammonia-N concentration were higher in the GS than FG treatments ($P < 0.05$). Inclusion of SAd, SFp and FPr did not influence these parameters in the FG.

The effects of FPr and its combination with SFp on CH₄ production might partly explain why cattle fed grass silage produce more CH₄ than cattle under pasture. The lack of difference in CH₄ production between FG and GS could be probably due to loss of volatile silage fermentation products during drying in preparation for the in vitro work.

373 An in vitro approach of two different carbohydrate sources incubated with different ruminal inocula

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Soybean hulls (**SH**) are a common energy supplement in ruminant diets. Evaluating their impact on methane emissions (**CH₄**) is crucial for designing sustainable feeding strategies that reduce greenhouse gases while maintaining efficient ruminal fermentation. This study aimed to examine the digestibility, CH₄, and ruminal environment (**RE**) of two substrates, corn grain (**CG**) and SH, containing both carbohydrate sources (starch or fiber), incubated with two ruminal inocula (**RI**) from ewes fed diets containing the alternative substrates (CGRI and SHRI). The in vitro experiment was conducted over three periods, with duplicates of sealed bottles for each treatment. A factorial design was used to evaluate the effects of incubated energy source (CG and SH) and RI origin (CGRI and SHRI), with RI taken from three lactating fistulated ewes fed annual ryegrass, CG or SH, and soybean expeller (isoproteic 16%CP), 16hs after feeding. The incubation involved 100ml-sealed bottles containing 0.2g of dry substrate and 40mL of buffered RI, maintained at 39°C in a water bath for 24hs. Digestibility (DMD, NDFD, ADFD), RE (pH, NH₃-N, and VFA), and CH₄ were analyzed using SAS ProcNLIN ($\alpha=0.05$). No interactions were found between substrates and RI for any variables. For substrates, DMD was higher in CG (855 vs. 758 g/kgDM, $P<0.0001$) but no differences were observed for NDFD or ADFD. Rumen inocula showed higher values in SHRI than CGRI for DMD (765 vs. 702 g/kgDM, $P=0.0002$), NDFD (396 vs. 275 g/kgNDF, $P=0.01$), and ADFD (330 vs. 179 g/kgADF, $P=0.02$). Higher pH was obtained in SH and SHRI (7.14 vs. 7.06, $P=0.0015$) compared to CG and CGRI. Acetate (**C₂**) concentrations were similar between substrates; SH had lower propionate (**C₃**: 3.01 vs. 3.86 mM, $P=0.02$), and butyrate (**C₄**: 1.10 vs. 2.13 mM, $P<0.0001$) than CG. No differences were observed for C₂/C₃ ratio. In SH, Total VFA (12.8 vs. 16.1 mM, $P=0.04$) were lower compared to CG, with CG showing a trend for higher VFAdm (2.78 vs. 3.46 mM/kgDM, $P=0.06$). No differences were observed for NH₃-N. For RI, SHRI had higher C₂ (11.6 vs. 8.73 mM, $P=0.01$) than CGRI, C₃ (3.1 vs. 3.78 mM, $P=0.06$) and C₄ (1.09 vs. 2.14 mM, $P<0.0001$) were higher in CGRI and C₂/C₃ ratio (4.68 vs. 2.51, $P<0.0001$) was higher in SHRI vs. CGRI. No differences were observed for total VFA, VFAdm, or NH₃-N. For CH₄/DM, CG showed higher values compared to SH (5.56 vs. 3.7 g/kgDM, $P=0.0287$) and in CGRI vs. SHRI (5.58 vs. 3.68 g/kgDM, $P=0.0248$). No differences were found for CH₄/DMD for substrates (GM: 6.62 vs. SH: 4.93 g/kgDMD, $P=0.1063$), but CH₄/DMD was 57% higher in CGRI (7.06 vs. 4.49 g/kgDM, $P=0.0154$). Average ruminal retention time exceeds 24hs, enhancing SH digestion without negatively affecting milk yield or quality compared to CG. These results are promising for SH as a sustainable energy supplement in dairy ewe diets, due to lower methane emissions and a 'healthier' rumen similar to pasture diets.

375 Impact of tannin-rich forages on biogas and methane emissions from sheep manure managed anaerobically

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Feeding ruminants tannin-rich forages has gained attention as a strategy to enhance nutrient utilization and mitigate greenhouse gas (GHG) emissions, particularly enteric methane emissions. However, limited research has explored the downstream effects of tannin-rich diets on methane emissions from manure. This study evaluated the biogas (CH₄ and CO₂) production from manure produced during sheep feeding trials with diets containing varying in vitro bioactivity (BIV) levels of tannin-rich forages. Manure was collected from sheep fed five diets: Calliandra calothyrsus with 7.5% BIV (CALO), Calliandra calothyrsus with 15% BIV (CAHI), Leucaena leucocephala with 7.5% BIV (LUEC), a combination of Calliandra calothyrsus and Leucaena leucocephala achieving 13% BIV (LECA), and a base diet with no BIV as a control (CONT). Sheep were fed these diets for at least two weeks before manure (a mixture of feces and urine) was collected for the biogas emissions study. The manure was mixed with inoculum (at a 2:1 volatile solids (VS) basis) obtained from an active anaerobic digester and incubated at 37°C with regular shaking in four replicates under anaerobic conditions. Other substrates, including inoculum and cellulose, served as blank and positive controls, with a total of 28 bath bottles incubated for a 62-day period. Biogas samples were collected from the headspace of the bottles, and headspace pressure was measured using a manometer. Gas samples were collected in evacuated vials using syringes for biogas composition analysis via gas chromatography (GC) method. At the end of the experiment, bottles were opened, and liquid samples were taken for VS reduction, fiber composition, volatile fatty acids (VFAs), and pH analysis. Results showed significant variations in biogas and methane production among the diets. The CONT manure produced the highest biogas (313.22 mL/g VS), while CAHI recorded the lowest (145.26 mL/g VS), reflecting a 53.62% reduction. CALO, LUEC, and LECA reduced biogas production by 26.82%, 37.28%, and 33.69%, respectively, compared to the control. Methane production followed a similar trend, with CAHI (80.59 mL CH₄/g VS) showing the lowest output and CALO (123.42 mL CH₄/g VS) the highest. In conclusion, tannin-rich forages, particularly CAHI with 15% BIV, demonstrated significant potential to reduce biogas and methane emissions from manure. Ongoing analyses of fiber composition, volatile solids reduction, pH, and volatile fatty acids will provide further insights into the observed emissions differences between treatments. These findings highlight the role of tannin-rich forages as a potential intervention for mitigating GHG emissions in livestock production.

395 Greenhouse gas emissions from manure of sheep fed on diets containing *Calliandra* and *Leucaena*

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Livestock emissions, mainly enteric methane (**CH₄**), and CH₄ and nitrous oxide (**N₂O**) from manure, account for about 11% of global greenhouse gas (**GHG**) emissions. In East Africa, *in vitro* studies showed that tannin-rich legumes, *Calliandra calothyrsus* and *Leucaena leucocephala*, significantly reduced CH₄ production. Subsequently, a study tested enteric CH₄ emissions in Red Maasai (tropical breed) and Dorper (subtropical breed) sheep fed diets containing *Calliandra*, *Leucaena* and *Calliandra* plus *Leucaena*. However, higher GHG emissions from manure of sheep consuming the test diets than emissions from manure of sheep on a control diet would partly offset any enteric CH₄ reduction associated with these legumes. Livestock breeds also influence manure N₂O emissions because of differences in nitrogen (**N**) excretion pathways in terms of ratio of dung-N to urine-N. Tropical breeds are often more efficient in retaining N, resulting in a greater proportion of dung-N. In contrast, temperate breeds tend to excrete more urine-N, which enhances manure N₂O emissions. This study measured CH₄ and N₂O emissions from manure of Red Maasai and Dorper wethers fed on five different diets in a 5 × 2 factorial design to evaluate potential trade-offs between manure and enteric emissions. The dietary treatments included *Calliandra* low (**CALO**; 16.7% *Calliandra*), *Calliandra* high (**CAHI**; 33.3% *Calliandra*), *Calliandra* + *Leucaena* (**LECA**; 21.0% *Calliandra* + 21.0% *Leucaena*), *Leucaena* (**LEUC**; 44.1% *Leucaena*), and a Control (**CONT**, soybean meal replaced the legumes). All diets had a forage-to-concentrate ratio of 80:20, the same crude protein (**CP**: 16%) and neutral detergent fibre (**NDF**: 44%) contents on dry matter (**DM**) basis. Fresh manure samples were collected from five Dorper and four Red Maasai wethers on each diet after 14-day adaptation period. Daily manure collections from each diet-breed combination were equally divided into two sets of three manure buckets, one set for manure sampling and another set for GHG measurements until each bucket had about 3 kg of fresh manure. The manure samples were incubated for four months with weekly adjustments of moisture content. Manure CH₄ and N₂O fluxes were measured daily during the first month and thrice per week during the subsequent three months using Los Gatos Research Gas Analyzer. Additionally, manure DM, organic matter, N, ammonium-N, nitrate-N, pH, carbon ©, and fiber fractions were analyzed in fresh manure and samples collected on 30, 60, 90, and 120 days. Results for the first 30 days of the study showed that the average manure CH₄ fluxes (mg CH₄-C kg⁻¹DM h⁻¹) were CALO (5.79), CAHI (23.24), LECA (20.09), LEUC (8.25), and CONT (5.39). The average manure N₂O fluxes (µg N₂O-N kg⁻¹DM h⁻¹) were CALO (131.80), CAHI (134.28), LECA (143.72), LEUC (6.22), and CONT (5.39). Whereas the average CH₄ fluxes from manure of Red Maasai (8.63) was lower than the fluxes from manure of Dorper (16.47) the reverse was true in relation to the average N₂O fluxes from manure of Red Maasai (129.12) and N₂O fluxes from manure of Dorper (40.20). Complete data for enteric CH₄ and manure GHG emissions will be available at the end of the experiment in May 2025.

396 Meta-analysis of methane variations explained by rumen accessible fatty acid, dietary, rumen, and animal variables

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Fat supplementation is a promising dietary strategy to mitigate methane (**CH₄**) emissions from dairy cows, although results across studies remain inconsistent. Factors like fatty acid composition and concentration, total fat concentration in the diet, physical fat form, and overall dietary characteristics have been observed to affect the inhibitory response of fats on CH₄ production. Using a meta-analytical approach, we quantitatively summarize variations in CH₄ emissions in lactating dairy cows in relation to dietary rumen accessible fatty acid (**RAFA**), dietary composition, animal characteristics, and ruminal fermentation. A database was collated from 48 publications containing 180 treatment means, which reported CH₄ emissions in lactating dairy cows fed additional fat. Dietary RAFA was calculated for C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 (*cis* and *trans* isomers), C18:2, C18:3 by multiplication of fatty acid intake predicted from NDS Professional model (RUM&N Sas, Italy) based on CNCPS 6.55 model with rumen fat accessibility indices. This index values depend on the physical form of the fat source which includes 4 levels: zero for rumen inert, low (0 to 0.3) includes whole cottonseed, medium (0.4 to 0.7) includes whole and rolled seed, and high (0.8 to 1.0) includes ground seed and oil. Linear mixed effect regression models were developed, where study was included as random effect in the model, and regressions were weighted by the inverse of the standard error of the observed mean. Models were compared using the concordance correlation coefficient, root estimated variance related to study and error, and corrected Akaike Information Criterion values. From the RAFA standpoint, most of the enteric CH₄ traits were affected by almost all RAFA including C12:0, C14:0, C16:0, C18:1 *cis* isomer, C18:2, and C18:3. Dietary starch and rumen available ether extract had negative slope both on daily CH₄ production and yield (expressed per unit of dry matter intake). Increasing dry matter intake and digestibility of neutral detergent fiber were associated with increased CH₄ production and yield. Ruminal variables also reflected the importance of rumen volatile fatty acids (**VFA**) in affecting CH₄ emission. Total VFA concentration, and molar proportions of acetate, and butyrate were positively related with CH₄ production, while molar proportions of propionate were negatively correlated with CH₄ yield and intensity (expressed per unit of energy-corrected milk). Model comparisons revealed that RAFA alone did not fully explain the observed variation in CH₄ emission. This suggests a more complex interaction among dietary fatty acids, other dietary composition, animal, and rumen variables. Notably, the inclusion of rumen fermentation variables improved model performance, as indicated by lower Akaike Information Criterion and higher concordance correlation coefficient values. These results emphasize the importance of considering these complex interactions to fully understand and effectively mitigate CH₄ emissions in dairy cows.

398 Performance and enteric methane emissions of sheep fed on diets containing *Calliandra* and *Leucaena*

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Ruminant livestock significantly contributes to anthropogenic greenhouse gas emissions, mostly through the production of enteric methane (**CH₄**). Therefore, mitigation of enteric CH₄ emissions from ruminant livestock is critical to limiting global warming. In East Africa, *in vitro* studies have shown that condensed tannin-rich forages, *Calliandra calothyrsus* and *Leucaena leucocephala*, significantly reduced the production of total gas and CH₄. These findings suggest the potential for incorporating *Calliandra* and *Leucaena* into ruminant diets to mitigate enteric CH₄ emissions. However, forages containing high levels of condensed tannins often are of low quality, poor acceptability, and many show more CH₄ reducing properties in *in vitro* trials than in *in vivo* experiments. The potentially negative consequences of these forages to animal nutrition and the uncertainty of their *in vivo* anti-methanogenic properties may relate to their inclusion levels in diets, interaction with other dietary components, and animal factors. The expected anti-nutrition properties and variation in the anti-methanogenic properties associated with these legume forage species complicates the *in vitro* efficacy claims. The objective of this study was to investigate the effects of including *C. calothyrsus*, *L. leucocephala*, and *Calliandra* plus *Leucaena* into a total mixed ration on enteric CH₄ emissions from sheep. The experimental diets included, on a dry matter basis (**DM**), the following five treatments (**T**): T1. *Calliandra* low (**CALO**; 16.7% *Calliandra*), T2. *Calliandra* high (**CAHI**; 33.3% inclusion of *Calliandra*), T3. *Calliandra* + *Leucaena* (**LECA**, 21.0% *Calliandra* + 21.0% *Leucaena*), T4. *Leucaena* (**LEUC**, 44.1% *Leucaena*), and T5. Control diet (**CONT**, soybean meal replaced the tested legumes as protein source). All diets had a forage-to-concentrate ratio of 80:20 on a DM basis and were balanced to have the same crude protein (**CP**: 16%) and neutral detergent fibre (**NDF**: 44%) contents. The study was a balanced randomized incomplete block design. In brief, 54 wethers comprising 30 Dorper and 24 Red Maasai sheep were stratified by breed and body weight into nine blocks of six wethers each. Four wethers within a block were randomly assigned to one of the four test diets and the remaining two wethers were assigned to a control diet. Animals within each block were split into two sub-blocks, each comprising three wethers (two wethers fed any of the four test diets and one wether fed a control diet) due to the limitation posed by three respiration chambers. After a 22-day adaptation period, enteric CH₄ production by each wether was measured in respiration chambers for 72 hours. Additional data collected were daily feed intake, body weight gain, and diet digestibility. Samples collected included rumen fluid for the analyses of volatile fatty acids and ammonia profiles, and urine and fecal samples for energy and nitrogen excretion. Data analyses will be complete by the end of May 2025.

402 Methane production from beef bulls under intensive feeding conditions measured with a Laser Methane Detector

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Although most cow-calf production systems in South Africa are subjected to extensive rangeland conditions, approximately 70% of all beef consumed in South Africa is produced from animals finished within intensive systems. The challenge is thus to produce enough beef for the expanding population while reducing greenhouse gas emissions and decreasing the negative environmental impact of intensive systems. Techniques for monitoring CH₄ at individual animal level is important to monitor methane mitigation alternatives while improving cattle production efficiency. Although not popular in livestock systems, the Laser Methane Detector (**LMD**) has demonstrated its viability to be utilized in enteric methane monitoring in ruminants from individual animals within their production environment. The study was aimed at measuring the methane yield of young Simbra (n=22), Pinzgauer (n=11), and PinZ²yl (n=9) bulls in an intensive feeding system. The study was conducted at the Irene experimental farm of the ARC-Animal Production Institute in South Africa. No more than 10 animals were housed in a 200m² enclosure and fed *ad lib* using individual Calan feed stations. Animals were fed a total mixed ration for 9 weeks where after methane measurements commenced. Feed intake was measured daily while the animals were weighed weekly. Methane measurements were taken from individual animals with a handheld LMD device. Each individual animal was herded into a corner at a radius of at least 6m away from any other animals when measured. The auxiliary targeting laser beam was then directed at the nostrils of the animal from a distance of approximately 3m. Five 60-second repeated measurements for each bull were taken twice a week over a three-week period at the same time of day (18h00). Methane concentration ppm-m (ml/m³) was converted to methane production (g/day) by making use of the model developed by Chagunda. Methane yield was calculated by dividing methane production (g/day) with daily feed intake (g/day). The experimental design was a completely randomized design with individual animals representing the three breeds as random replicates. The data collected were subjected to analysis of variance (**ANOVA**) to test for a significant breed effect using the **GLM** (General Linear Models) Procedure of SAS software (Version 9.4). Fisher's least significant differences (**LSD's**) were calculated at the 5% level to compare means for significant breed effects. Pearson's correlation coefficients were also calculated to examine the data for significant linear relationships. Results from the study showed no significant differences in methane yield (g/day) between the three breeds: Pinzgauer 7.49 ± 1.73, PinZ²yl 7.41 ± 0.47, and Simbra 7.10 ± 1.37. There was no significant correlation between methane yield and either end weight, feed efficiency, average daily gain, or daily feed intake. A correlation was found, however, at the 10% level, between methane production (g/day) and end weight (P = 0.08) as well as daily feed intake (P=0.07).

406 Multi-objective feed formulation for minimizing cost and methane emissions in dairy production

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Enteric methane emissions represent a significant source of greenhouse gases in dairy production. Dietary mitigation strategies have been widely explored, yet research on the associated costs remain limited. In this study, we formulated a multi-objective feed formulation model aimed at simultaneously minimizing 3 objectives, 1) diet cost, 2) deviation from nutrient requirements, and 3) enteric methane emissions. A vectorized implementation of the NASEM 2021 model was coded in Python and the multi-objective optimization problem was solved using Non-dominated Sorting Genetic Algorithm (**NSGA-III**) via the Pymoo package. To demonstrate model application, a lactating Holstein cow was considered as follows: 611 kg body weight, 27 kg/day milk production, 4.1% milk fat, and 3.4% milk protein, 100 days in milk and 30 days pregnant, with a body condition score of 3.5. A set of 33 feed ingredients were selected from the NASEM 2021 feed library and prices were sources locally (Davis CA). Feed allocation was constrained to ensure neutral or positive nutrient balances, with a minimum dietary neutral detergent fiber of 190 g kg⁻¹ DM, and maximum dietary starch, fatty acid, and ash levels of 300 g kg⁻¹ DM, 70 g kg⁻¹ DM, and 100 g kg⁻¹ DM, respectively. The evolutionary algorithm parameters were as follows: a maximum of 1,000 generations, simulated binary crossover (probability = 0.9, η = 5), and polynomial mutation (probability = 0.3, η = 5). An adaptive ϵ -constraint method was employed for constraint handling. The total computing time was 35.1 seconds on a standard workstation (Intel Core i7, 32 GB RAM). The model generated a Pareto front of 26 non-dominated solutions in a single run, allowing evaluation of trade-offs between methane emissions and diet cost without pre-defined penalties or priorities. Among these, the least cost and lowest methane emission diets were priced at \$4.20 and \$5.60 per cow per day, respectively, with corresponding methane yields of 18.0 and 17.2 g/kg DMI. All modeled methane yields were lower than the intercontinental average (20.1 g/kg DMI) reported by Niu et al., 2018. Diets associated with lower methane emissions tended to contain less NDF (300 g/kg DM) and higher ether extract content (67 g/kg DM). Access to the complete Pareto front facilitates comprehensive evaluation of cost-methane trade-offs, supporting informed decision-making. Future research will compare nutrient requirement models and evaluate model performance across various diets, animal categories, and management practices, assessing impacts on both production and environmental outcomes.

410 Strategic screening of forages from international genebanks for low enteric methane emission

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Mitigating methane emissions from ruminant livestock is a key challenge in reducing agricultural greenhouse gas contributions. While numerous strategies have been explored, leveraging plant diversity from global south gene banks presents an untapped opportunity. This study examines the potential of forages from three international gene banks (CIAT, ILRI, and ICARDA) to reduce methane emission while maintaining or enhancing livestock productivity. A total of 172 forage samples representing a wide diversity of species and accessions (categorized as 88 legumes, 28 grasses, 25 browses and 31 others) were evaluated for their effects on rumen fermentation, methane production, and degradability using an in vitro rumen simulation method based on rumen liquor collected from Friesian × Jersey cows, forage samples were incubated for 24 hours. A decision-making tool was developed using Microsoft, Excel Software to evaluate and select forages based on the parameters of interest, which were CH₄ intensity i.e. ml CH₄/g dry matter degraded (**DMD**) and percent DMD. Forages were ordered from highest to lowest for the parameters of interest. The objective has been to select forages that produced low ml methane/g DMD and high percent DMD. Therefore, as a first step, the average of the highest 10% of the values was taken for the former parameter (these forages are of least interest since they gave the highest values), and values for rest were expressed as percentage of the average. Out of 88 legumes, 15 forages that have the least values for ml methane/g DMD were selected. In the second step, values for the percent DMD were listed against the selected 15 forage samples. Out of these 15 forage samples, promising candidates were identified that had low methane production per unit DMD and high percent DMD. Using this approach the promising forages identified are: *Hedysarum carnosum*, *Hedysarum coronarium*, *Vicia narbonensis*, *Trifolium burchellianum*, *Grona heterocarpa*, *Centrosema schottii*, *Codariocalyx gyroides*, and *Desmodium intortum*. For these forages methane production as ml methane/g DMD ranged from 18.4 and 44.4. The average of 10% of the top values for this parameter was 67.6 ± 2.25 (mean $\pm$ SD, n = 9), suggesting that the identified forages produce 34.3% to 72.7% lower methane than those that produce high methane per unit DMD. Likewise, percent DMD for the lowest 10% was 47.7 ± 6.76 (mean $\pm$ SD, n = 9). For the identified 15 forages, the percent DMD were 87.1% to 101.8% higher compared with the average of 10% of the lowest values for this parameter. The evaluation and ranking, separately of grasses and herbaceous and browse legumes are in progress using the approach. Similarly, acetate/propionate ratio of the samples of least interest (having high methane production per unit DMD) and those of the promising forages identified using the approach is being analyzed. The selected forages form potential candidates for metabolomic profiling to confirm the presence or discovery of anti-methanogenic compounds and in vivo evaluation.

416 Feeding practices and socio-economic drivers of dairy productivity in Tamil Nadu: insights from smallholder systems

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Livestock farming is integral to rural livelihoods in India, particularly for smallholder and landless households. It contributes around 30% to rural household incomes, provides substantial employment opportunities, and promotes equitable income distribution, often surpassing earnings from crop cultivation. However, feed scarcity – both in quality and quantity – poses a critical challenge to livestock productivity. This study investigates the factors contributing to low dairy productivity in Tamil Nadu, with a specific focus on feed management practices and their implications for optimizing milk yield and promoting climate resilience. Optimizing dairy productivity in this context refers to achieving daily yields of 8–10 liters per lactating animal, while ensuring financial viability and reducing emission intensity (kg CO₂e per liter of milk produced).

Using the Feed Assessment Tool (FEAST), the research systematically analyzed feeding patterns, resource availability, and institutional gaps. Participatory Rural Appraisal methods, including focus group discussions and key informant interviews, were conducted across six districts spanning three agro-climatic zones. The study engaged 180 farmers and eight stakeholders – comprising feed entrepreneurs, researchers, and academicians.

Findings revealed significant socio-economic and agro-climatic disparities. Landless households made up 24% of the surveyed population, reaching a peak of 46% in Karur. Fallow lands, underutilized due to insufficient rainfall, were common in Dharapuram and Karur. Dairy farming was prevalent across all landholding categories, with landless laborers in Pollachi relying heavily on livestock as a primary income source. On average, livestock contributed 39% to household incomes, peaking at 46% in Dindigul. Feeding practices highlighted a heavy dependence on purchased feed – such as concentrates, paddy straw, and cotton gin trash – which accounted for over 34% of dry matter and more than 50% of crude protein intake. Higher milk yields (up to 9.1 liters per lactating cow per day) were recorded in Erode, Dindigul, and Salem, corresponding with higher reliance on external feeds. However, this reliance imposed significant financial burdens, with average feed costs exceeding ₹3 lakhs per household and peaking at ₹5.9 lakhs in Erode. Despite its potential to lower costs and reduce GHG emissions, improved fodder cultivation remained limited, with households allocating only 0.1 to 0.18 hectares on average. Limited access to quality fodder seeds further hindered the adoption of climate-smart feeding practices. Targeted interventions – such as promoting high-yielding fodder varieties, optimizing feeding regimes, and enhancing institutional support – are essential to increase milk productivity while reducing emission intensity. Encouraging decentralized entrepreneurship can also improve access to affordable inputs and advisory services. This study highlights the importance of aligning technological solutions with local resource contexts and institutional capacities to enhance both dairy productivity and environmental sustainability. Such strategies are vital for creating a climate-resilient livestock sector that ensures rural income security while minimizing ecological footprints.

417 Evaluating the impact of seaweed supplementation on in vitro digestibility and methane mitigation

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We evaluated the effects of supplementing various seaweed species on in vitro digestibility parameters and methane production. Four seaweed species were tested: *Saccharina latissima* (sugar kelp), *Sargassum natans* and *Sargassum fluitans* blend, *Sargassum horneri* (devil weed), and *Macrocystis pyrifera* (giant kelp). We hypothesized that, regardless of the dose, seaweed inclusion would decrease methane emissions from rumen fermentation. The seaweed species were dried, ground, and included in rations at varying inclusion levels (0%, 1%, 5%, and 10% of dry matter for *S. horneri* and the *Sargassum* blend, and 0%, 5%, and 10% for *M. pyrifera* and *S. latissima*). The seaweed replaced equal proportions of corn silage and grain mix in the corn silage-based lactation total mixed ration (60% corn silage, 40% grain mix). Dry matter digestibility (**DMD**), neutral detergent fiber digestibility (**NDFD**), and methane emissions were measured. Total mixed ration substrates (4 mm; 0.70 g per F57 bag) were incubated with buffered rumen fluid for 24 hours, with six replicates per run. Data were analyzed using the GLIMMIX procedure of SAS. The statistical model included treatments as fixed factors, and differences were declared significant at $P \leq 0.05$. The inclusion of *Sargassum horneri* at 5% and 10% resulted in a reduction in DMD and NDFD, with a corresponding decrease in methane emissions. *Saccharina latissimi* inclusion in the diets decreased DMD and NDFD only at higher inclusion levels (10%), where methane reduction was also observed. For the *Sargassum natans* and *Sargassum fluitans* blend, methane emissions were lowered at 10% inclusion, with no significant effects on digestibility at 1% and 5%. *Macrocystis pyrifera* supplementation improved DMD at 5% compared to the control, while methane emissions were decreased linearly in a dose-dependent manner. These findings indicate that certain seaweed species have the potential to mitigate methane emissions without significantly compromising digestibility, depending on the species and inclusion level. However, higher inclusion levels may negatively impact fiber digestibility in some species. This study highlights the importance of species-specific and dose-dependent evaluations to optimize the use of seaweeds as a methane mitigation strategy in ruminant nutrition. Further research is recommended to explore the underlying mechanisms and practical applications in livestock systems.

426 Associations between carcass, production traits and methane emissions in beef cattle

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As global demand for meat continues to rise and environmental concerns intensify, the livestock industry faces mounting pressure to balance productivity with sustainability. In particular, enteric methane (**CH₄**) reduction is a key target in climate change mitigation efforts. Understanding how key production traits relate to CH₄ emissions is essential. This study examined how key production traits such as: carcass conformation and fat score (assessed via the EUROP classification system), carcass yield, and average daily weight gain (**ADWG**), relate to CH₄ emissions. Data were collected from five annual cohorts (2019–2023) of beef cattle ($n = 433$, consisting of 150 females and 283 males), each monitored during its first winter housing period. Upon housing, cattle averaged 249 ± 25 days of age and 327 ± 48 kg live weight. Methane output was measured using a GreenFeed system and expressed as total grams of CH₄ per day and grams of CH₄ per kilogram of metabolic weight ($BW^{0.75}$). Conformation and fat scores were converted to an ordinal scale. Statistical analyses employed Kendall's tau (τ) for the ordinal traits and Pearson's correlation r for continuous traits (carcass yield, ADWG). Although most associations were statistically significant, they were weak in magnitude. Conformation and fat score showed small positive correlations ($\tau \approx 0.10$ – 0.19) with total daily CH₄ but only marginal or non-significant relationships with CH₄ per kilogram of metabolic weight. Carcass yield was uncorrelated with CH₄ per kilogram of metabolic weight ($r = -0.03$, $p = 0.48$) but demonstrated a weak positive association ($r = 0.12$, $p = 0.013$) with total daily emissions. While ADWG exhibited a moderate correlation with daily CH₄ ($r = 0.28$, $p < 0.0001$), it too was unrelated to CH₄ per kilogram of metabolic weight ($r = 0.07$, $p = 0.14$). These findings suggest that higher conformation scores and faster growth rates tend to coincide with greater total methane production, but the effect on methane intensity (when adjusted for metabolic weight) is limited or non-significant. Given the weak magnitude of most associations, these results should be interpreted with caution. Further research is needed to clarify how beef production traits might be managed to balance productivity goals with the ongoing need to mitigate greenhouse gas emissions.

428 Effect of the combination of different feed additives (3NOP, monensin and nitrate) on rumen fermentation

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In the rumen, methanogens serve as the major hydrogen (**H₂**) sink and this interdependency for H₂ between methanogens and other fermenting microbes drives fermentation of feeds. It has been reported that reduced methanogenesis by directly inhibiting the activity of methanogens increases H₂ concentrations which may result in an increase in partial pressure of H₂. Although the literature reports a significant increase in alternative metabolic H₂ sinks and dissolved H₂ in ruminal contents of animals supplemented with methanogens inhibitors, these increases were not able to account for H₂ spared under inhibited methanogenesis. Therefore, there is still a need to understand how alternative H₂ sinks that can compete with methanogens for H₂ under inhibited methanogenesis work.

This study aimed to evaluate the effect of combining three additives with different mode of action to reduce methanogenesis: a direct inhibitor of methanogenic archaea (3-Nitrooxypropanol, **3NOP**), an alternative H₂ sink (nitrate) and an additive that decreases H₂ availability in the rumen (monensin). An in vitro experiment was conducted at separate sites (SRUC in Scotland and CSIC in Spain), using different rumen donor animals (cattle or goats), and substrates for the incubation (silage-based, concentrate-based). The treatments included: 3NOP (100 mg/kg DM and 600 mg/kg DM), nitrate (30 g/kg DM) and monensin (90 mg/kg DM) and combinations of these three additives (3NOP + Nitrate, 3NOP + monensin, Nitrate + monensin, and all 3 additives together). Rumen fluid from 4 animals (n=4) was mixed 1:2 with a buffer solution (50 mL) and incubated in Wheaton bottles with 500 mg of either diet and the additives for 24 hours at 39 °C. At the end of the incubation (24h), gas production was measured and samples were collected for methane (**CH₄**), H₂ and volatile fatty acids (**VFA**) analysis.

Consistent effects were observed between both sites. 3NOP decreased CH₄ by 60% at 100 and 600 mg/kg DM with cow and goat rumen fluid as inoculum, respectively. The combination of the three additives achieved greater reductions, decreasing CH₄ by 70-77% and 82-93% with goat and cow rumen fluid, respectively. Methane reduction was more pronounced with the silage-based diet as compared to the concentrate-based diet. As expected, an increase in H₂ was observed with 3NOP (20%); however, the combination of the three additives caused a decrease in the accumulation of H₂, bringing levels closer to those of the control treatment. A slight decrease in VFA (3%) was also observed with all treatments in incubations with rumen fluid from goats, but it was not significant with the silage diet. This reduction was more substantial in incubations with rumen fluid from cows (10%). This study highlights the potential of combining additives with distinct modes of action to achieve substantial CH₄ reductions. Furthermore, it provides a framework for exploring how H₂ spared from decreased CH₄ production could be redirected within rumen fermentation pathways. Long-term in vitro studies are underway to confirm these findings and assess associated microbial shifts.

432 Improved productivity and enteric methane following molasses block supplementation of Indonesian smallholder dairy cattle

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Smallholder dairy farming is an important component of Indonesia's agricultural sector, particularly in Java where over 98% of the nation's dairy cow population are located. These smallholder farms typically maintain herds of between 2-20 cows, contributing substantially to local economies and livelihoods. Average milk yields are below international standards, with cows often producing less than 10 litres per day. As methane mitigation strategies are urgently required to help reduce the environmental impact of dairy farming in Indonesia, this study investigated the impacts of a molasses-based lick block nutrition supplement on Indonesian smallholder dairy farm milk production, reproduction and enteric methane. A shipment of "Emission Control Blocks" (ECBs), which are molasses-based supplement is a proprietary formulated lick block containing naturally occurring plant-based methane inhibitors, were donated and shipped to Indonesia by the Australian company AgCoTech. A trial was conducted on two dairy farms in East Java, across Malang and Tulungagung. The trial commenced on the 25th of April 2024 and ran for a period of 15 weeks. Two farms participated in the trial. A total of 85 cattle were enrolled across both farms and evenly placed into treatment (ECB) and control groups. Animal data was recorded, along with animal weight, milk production, methane output using a Methane mini laser, and health events were recorded throughout the trial. Preliminary analyses of the data indicated treatment cattle increased in body weight by 4.9kg more than control cattle over the duration of the trial. On a weekly basis, the average milk production by volume is 31.9% higher in treatment cows. The proportion of dairy cattle with no ECB treatment that became pregnant was 23% compared to 58% of the treatment group recording a successful pregnancy, indicating an improvement in pregnancy rate by a factor of 2.5. For the IPCC model, this was estimated as 30 and 75% annualized pregnancy rate. Based on an assumed lactation of 200 days, the extra 3.5 l/d achieved in treatment cattle, and 6,500 IDR per l, each cow provided an ECB provided an extra income of 4,550,000 IDR. This equates to USD \$284.02 per cow in value of extra milk. The IPCC calculations indicate the ECB led to a 35% reduction in daily methane production. On analysis of raw (non-transformed data), treatment cattle produced more methane as measured by the mini laser. However, for every litre of milk produced, cattle on ECBs produced between 19-28% less methane. This indicates that the ECB improves the efficiency of milk to methane production.

The authors confirm that the research to be reported will be completed, and shared at the time of the conference.

433 Potential use of red seaweed *Gracilaria* spp. to mitigate enteric methane emissions in beef cattle

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Enteric methane (CH_4) in ruminants is associated with an increase in greenhouse gas emissions and a loss of dietary gross energy, which impacts production efficiency. Red seaweed is of interest due to bromoform, a chemical with the ability to decrease enteric CH_4 emissions in ruminants. Supplementing beef cattle diets with algae may promote sustainable beef production by enhancing cattle productivity and potentially mitigating enteric CH_4 emissions. This study evaluated the effects of different inclusion levels (0, 1, 5, and 10%) of red seaweed *Gracilaria* spp. on in vitro fermentation parameters using ruminal fluid from two cannulated animals. Experiment 1 utilized a randomized complete block design (RCBD) with three replicates. Fixed effects were algae, inclusion level, and drying method, with incubation day as a random effect. Experiment 2 employed a RCBD with fixed effects of algae and inclusion level, and incubation day as a random effect. Polynomial contrasts (linear, quadratic, and cubic) were applied in both experiments. In experiment 1, *G. mammillaris* (GM) and *G. damicornis* (GD) were evaluated under two drying methods, sundried (SD) and freeze dried (FD). For pH, a positive linear effect was observed ($P < 0.01$) for GMSD, GDSD, and GDFD, and a quadratic effect ($P < 0.03$) for GMFD. A negative linear effect of algae inclusion was observed ($P = 0.05$) in GMFD and GDSD for CH_4/g of dry matter (DM) incubated, and in GDSD for total volatile fatty acids (mM; $P = 0.04$). An interaction between algae spp. and drying method was observed for acetate molar proportions (MP; $P = 0.04$), with a linear increase for GMFD and GDSD ($P = 0.01$ and $P = 0.04$ respectively). Propionate MP decreased linearly for GMFD ($P = 0.01$) and the acetate to propionate ratio was higher for GMSD ($P = 0.04$) and GMFD ($P < 0.01$). Experiment 2 evaluated three *Gracilaria* spp.: *G. mammillaris* (GM), *G. damicornis* (GD), and *G. coronopifolia* (GC). Algae were snap-frozen with liquid nitrogen and subsequently freeze dried, prior to incubation. A negative linear effect in algae inclusion level was observed in total gas produced for GD ($P = 0.03$) and GC ($P = 0.05$). A linear decrease was observed in CH_4/g of DM incubated for GD ($P = 0.01$) and GC ($P = 0.01$). This decrease persisted for CH_4/g of OM fermented ($P = 0.05$) in both GD and GC. Acetate MP increased linearly with algae inclusion level for GD ($P = 0.03$), whereas propionate MP linearly decreased in GC ($P = 0.03$), and a cubic effect was observed for GD ($P = 0.04$). Bromoform had no correlations with variables according to Pearson correlation coefficients. Inclusion of the red seaweed *Gracilaria* spp. has the potential to reduce CH_4 production; however, this reduction was not correlated with bromoform concentrations measured in the algae. Other compounds may have affected the fermentation, and they warrant further investigation. Snap freezing resulted in greater bromoform concentration regardless of drying method, indicating that harvesting method is an important aspect to manage during red algae production for CH_4 mitigation.

439 Ginger powder meal reduced methane gas emission from three traditional grasses used in ruminant production

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The energy lost as methane from the ruminal fermentation of highly fibrous feeds like tropical grasses is an environmental concern that requires urgent attention, since the release of methane gas has been implicated as one of the greenhouse gasses contributing to the global warming effect. To cut down on methane emissions from ruminal fermentation processes, this would require the design of strategies that would reduce the volume of methanogenic bacteria, which are responsible for methane production in the rumen. Natural additives contain phenolic compounds that are notable for cutting down on methane output without significant trade-off effects. This study therefore evaluated the inclusion of ginger powder levels as a feed additive for grasses on the *in vitro* gas and methane production levels. The treatments comprised three tropical grasses (*Brachiaria decumbens*, *Cenchrus purpureus* and *Megathyrsus maximus*) and two ginger powder inclusion levels (0 and 5 mg/g dry matter (DM) in a 3 x 2 factorial experiment which was laid out in a Randomized Complete Block Design. The grasses were harvested in their 2nd growing seasons on the 4th week of July, 2023, at 8 weeks after re-growth. The harvested grasses were dried and milled. Fresh ginger rhizomes were washed, air dried till they attained constant weight, were milled using a hammer mill, and passed through a 1 mm sieve screen. Afterward, the grasses were mixed with or without the ginger powder based on the treatment combination. Rumen fluids were collected from slaughtered cattle that had been fasted over-night, was filtered through 6-layers of cheese cloths, and mixed with a rumen-buffer solution in the ratio 2:1. *In vitro* gas production test was performed by incubating 200 mg DM of the substrates (based on the treatments) for 24 h with 10 replicates per treatment in a single run. At the end of the incubation period, the total gas volume, methane gas produced, and dry matter digestibility were recorded. The inclusion of 5 mg/g of ginger powder significantly ($p < 0.05$) raised the total gas volume produced *B. decumbens* (30.65 ml/200mg DM) and *C. purpureus* (28.33 ml/200mg DM), but significantly ($p < 0.05$) lowered the total gas volume produced by *Megathyrsus maximus* (17.50 ml/200mg DM). The relative methane gas volumes as a proportion of the total gas produced were significantly lowered for *B. decumbens* (16.98% reduction) and *C. purpureus* (11.22% reduction) with the inclusion of ginger powder; however, no significant changes were observed for *Megathyrsus maximus*. Also, the ginger additive had no significant ($p > 0.05$) effect on the *in vitro* dry matter digestibility. The results suggest that the effect of 5 mg/g of ginger powder inclusion was grass-species dependent; however, its effect for lowering methane gas production was evident. This study recommends the testing of impact of higher inclusion levels of ginger powder to further evaluate its associative effects with the evaluated grass species.

441 Inclusion of *Parquetina nigrescens* leaf-meal lowered methane gas emission from cassava residue-based diet

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Enteric methane production is a significant concern in ruminant nutrition, resulting in the loss of carbon sources and inefficient energy utilization. This phenomenon accounts for a substantial loss of 8-14% of digestible energy intake and contributes notably to greenhouse gas emissions and global warming. *Parquetina nigrescens* leaves have been found to contain various phytochemicals, including alkaloids, flavonoids, saponins, and tannins, which possess antimicrobial properties. These properties make *P. nigrescens* a potential additive for mitigating methane production in ruminants. The objectives of this study are to determine the effect of varying inclusion levels of *P. nigrescens* leaf meal (PNLM) on *in vitro* dry matter degradation, total gas and post incubation parameters such as estimating the organic matter digestibility, metabolizable energy and net energy values of substrates containing varying inclusions levels. The experimental feed substrates used for *in vitro* studies consisted of 50% cassava peel, 20% cassava leaves, 20% *Megathyrus maximus* and 10% concentrate on dry matter basis. *Parquetina nigrescens* leaf meal was included in the substrate at varying levels of 0 (control), 1, 2, 3, 4, and 5%, making six treatments set up as a completely randomized design. *In vitro* gas production technique described by Menke and Steingass was conducted over a 48-hour period and data were collected total gas, methane, dry matter digestibility, ammonia-nitrogen, volatile fatty acids, and post-incubation estimates. Data obtained were statistically analysed using one-way analysis of variance. The result showed that the inclusion of PNLM had ($p < 0.05$) significant effect on gas production from the 24th to 48th hours of incubation while there were no significant ($p > 0.05$) effects at early periods of incubation. The inclusion levels of PNLM at 4% and 5% showed significant ($p < 0.05$) effect on when *in vitro* gas production parameters compared to the control and 1% inclusion levels. The inclusion of 2 - 5% PNLM in the substrate degraded caused a reduction ($p < 0.05$) of *in vitro* total gas (16.80 – 10.80ml), methane (6.21- 3.53%) and ammonia nitrogen (20.14 -15.15 mg/100ml) production, *in vitro* dry matter degradation (66.37 – 54.14%). However, inclusion of PNLM at 3 - 5% increased ($p < 0.05$) total volatile fatty acids (36.70 – 54.90 mM/ 100 ml), while the organic matter digestibility, metabolizable energy and net energy were not affected ($p > 0.05$). The study concluded that including 3-5% *P. nigrescens* leaf meal in the ruminant diets could effectively reduce methane and ammonia degradation in the rumen. Additionally, including *P. nigrescens* leaf meal at 4-5% of could reduce *in vitro* dry matter digestibility, likely due to its antimicrobial activity. These findings suggest that *P. nigrescens* leaf meal has potential as a feed additive for improving ruminant nutrition and reducing environmental impact.

443 Optimizing sheep diets with agroindustrial by-products: Effects on fermentation, degradability, and methane emissions

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The use of agro-industrial by-products that are not edible for humans in ruminant diets is a strategy to reduce food competition between humans and animals, minimize the environmental impact of waste disposal, and promote the circular economy. This study evaluated three diet formulations for sheep, comparing a conventional diet (Diet 0 – Tifton hay + *Tithonia diversifolia* + soybean + corn) with alternative diets composed of agro-industrial by-products (Diet 1 – Tifton, *Tithonia diversifolia*, cassava bagasse, cottonseed meal, and orange bagasse; Diet 2 – Tifton, *Tithonia diversifolia*, sunflower meal, and cassava bagasse; Diet 3 – Tifton, *Tithonia diversifolia*, brewer's yeast, ground canola, and orange bagasse). All diets were formulated to be isoproteic and isofibrous. Organic matter degradability, methane production, and the partitioning factor were evaluated in a 24-hour in vitro assay, considering the progressive inclusion of by-products (25%, 50%, and 100% of the concentrate's dry matter, replacing corn and soybean). The optimal substitution level was determined through regression analysis and parameter performance. The results showed that in vitro organic matter degradability was highest in Diet 3 (566 g kg⁻¹), significantly differing from the others ($P < 0.01$). With increasing by-product inclusion, degradability rose up to 50% (540 g kg⁻¹), showing a slight reduction at 100% (505 g kg⁻¹). Total gas production was also highest in Diet 3 (55.9 mL g⁻¹ of organic matter), while by-product inclusion increased gas production up to 25% (53.2 mL g⁻¹), followed by a decrease at higher inclusion levels (47.2 mL g⁻¹). Methane (CH₄) production followed a similar trend, being highest in Diet 3 (4.62 mL g⁻¹ of organic matter). On the other hand, the partitioning factor, which assesses fermentation quality by relating gas production to organic matter degradability, was significantly higher in Diet 3 (3.37), progressively increasing up to 50% inclusion of by-products, indicating greater efficiency in microbial biomass production. Although Diet 3 generated the highest methane production in absolute terms, this should not be interpreted solely as a negative outcome. Higher methane values in this context reflect more efficient fermentation, with improved substrate degradability and a lower gas yield per unit of organic matter degradability. This is further supported by the significantly higher partitioning factor observed for Diet 3, indicating a more efficient conversion of nutrients into microbial biomass by the ruminal microbiota. Diet 3 demonstrated the highest organic matter degradability, with values improving as the inclusion of by-products increased up to 50% level, followed by a slight decline at higher levels. Notably, fermentation efficiency – reflected by the partitioning factor – was also greatest in Diet 3. These findings suggest that Diet 3, which includes up to 50% agro-industrial by-products in the concentrate's dry matter, achieves an optimal balance between degradability and fermentation efficiency. Despite the higher methane output, this formulation promotes better nutrient utilization and microbial biomass yield, enhancing productivity while supporting more sustainable use of by-products in ruminant diets.

446 Growth, methane emissions, and nutrient utilization in buffaloes consuming regionally important diets across the Philippines

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This study evaluated the effects of four regionally important feeding regimes on growth performance, nutrient utilization, and methane emissions of growing buffaloes in the Philippines. Twelve buffaloes were randomly assigned to four diets, formulated using regionally available feed ingredients and designed to achieve a target average daily gain (**ADG**) of 500 g/d. The diets were as follows: (**T1**) 85% Napier grass with 15% Rice Straw, (**T2**) 100% Napier grass, (**T3**) 100% mixed grasses (Paragrass, Mulato, Mombasa), and (**T4**) 92% Napier grass with 8% *Leucaena leucocephala*. The results revealed significant variations in growth performance, nutrient digestibility, and methane emissions across the dietary treatments. Buffaloes fed the mixed grasses diet (T3) achieved the highest growth performance, with an ADG of 792.79 g/day, surpassing T2 (612.61 g/day), T4 (648.65 g/day), and T1 (531.53 g/day). Additionally, T3 exhibited superior nutrient digestibility, with Dry Matter Digestibility (**DMD**) at 75.47% and Crude Protein Digestibility (**CPD**) at 80.03%. Feed intake was highest in buffaloes on the Napier grass with *Leucaena* diet (T4), whereas T3 demonstrated the most efficient feed utilization, with balanced Dry Matter Intake (**DMI**), Organic Matter Intake (**OMI**), and Concentrate Intake (**CPI**). In contrast, T1 had the lowest feed intake and DMI. From an economic perspective, T3 proved to be the most cost-effective diet. It recorded the lowest total feed cost (Php 1,067.67), daily feed cost (Php 35.59), and cost per kilogram of gain (Php 45.36). Moreover, T3 had the lowest Feed Conversion Ratio (**FCR**) of 9.91 kg, indicating high feed efficiency relative to growth performance. Methane emissions were lowest for the 100% Napier grass diet (T2), which recorded the lowest average daily methane output (57.72 g/day). It also demonstrated reduced methane emissions per unit of DMI, OMI, and CPI, making it the most environmentally sustainable feeding strategy among the diets studied. When the buffaloes reached a uniform bodyweight at 400 Kg, the total estimated methane emissions were lowest for T3 (8.28 kg), followed by T2 (9.64 kg), T4 (10.13 kg), and T1 (11.25 kg). These findings highlight the trade-offs between maximizing growth performance and minimizing environmental impact in buffalo production systems. The mixed grasses diet (T3) demonstrated the best outcomes in terms of growth, nutrient digestibility, and economic viability, making it a suitable choice for improving productivity. However, the 100% Napier grass diet (T2) emerged as the most effective strategy for mitigating methane emissions, highlighting its potential for reducing the environmental footprint of buffalo production. This study underscores the importance of regionally adapted feeding strategies to balance productivity and sustainability in buffalo production systems. Future research should focus on optimizing diet formulations to enhance buffalo productivity while mitigating greenhouse gas emissions, ensuring sustainable and eco-friendly livestock production in the Philippines.

450 Meta-analysis of dietary tannins in small ruminant diets: effects on intake, digestibility, and ruminal fermentation

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Plants naturally produce tannins, which are secondary metabolites that serve as protective mechanisms. In animal nutrition, these compounds play a complex role, functioning as both antinutritional elements and bioactive substances. They have the potential to influence nutrient metabolism and enhance product quality. This meta-analysis aimed to evaluate the effects of dietary tannins on nutrient intake, digestibility, and fermentation. Ninety-seven studies were included in this meta-analysis. The effects of tannins were analyzed using a random-effects model to determine the weighted mean difference between treatments with tannins and the control (without tannins). Publication bias and heterogeneity between studies were explored, and meta-regression and subgroup analyses were performed. The results indicated that tannin significantly increased the intake of dry matter, but not crude protein (**CP**) and neutral detergent fiber (**NDF**). Dietary tannin reduced digestibility of dry matter, organic matter, NDF, and ADF, but not cellulose and hemicellulose. Interestingly, tannins in the diet of small ruminants reduced the emission of the greenhouse gas emissions including NH³-N and CH₄. Regarding short-chain fatty acids, dietary tannins reduced butyrate, but had no impact on acetate, propionate, and acetate: propionate. In conclusion, the results highlight the dual role of tannins as bioactive compounds with potential environmental benefits.

451 Chemical composition and in vitro digestibility of *Sulla flexuosa* (L.) Medik. ecotypes in Mediterranean climate

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The present work was part of assessing wild genetic plant resources of forage interest in Northern Morocco and aimed to study the agro-morphology and nutritional value of *Sulla flexuosa* (L.) Medik. (*Hedysarum flexuosum* L.) ecotypes. The seeds of twenty-one wild *S. flexuosa* (L.) Medik. ecotypes were collected from 21 sites. These 21 ecotypes were cultivated in three completely randomized block designs for two consecutive years. The ecotypes were collected at the budding, start of flowering, and full-flowering stages. The plants were cut at three stages, and nutritive value analyses were conducted, including dry matter, organic matter (OM), crude protein (CP), ether extract, and fibers (NDF, ADF, and ADL). The in vitro dry matter and organic matter digestibility of the plants was determined using an enzymatic technique (with pepsine and cellulase), and rumen fluid was determined using a DAISY II apparatus. CP digestibility was assessed using protease enzymes. With the advancement of the phenological stage of the plants, there was a significant increase in ether extract (+ 22.5%), ADL (+ 20.0%), ash (+ 14.0%), NDF (+ 11.9%), and ADF (+ 7.9%) contents, as well as an equally significant decrease in metabolizable energy (ME, -19.3%) and in the OM (-9.8%), CP (-2.8%), and true (-12.5%) digestibilities, showing a possible decrease in greenhouse gases with advancing maturity stages. *Sulla flexuosa* (L.) Medik. ecotypes showed little increase in crude fiber CF (+ 3.0), CP (+ 2.3%), and ether extract content (+ 2.4%), and a small decrease in NDF (-4.2%), ADF (-0.7%), and ADL (-1.8%) content between the two years. In Vitro True Digestibility (IVTD) exceeded the enzymatic digestibility of OM. This discrepancy may be attributed to the experimental approach, wherein the actual digestibility incorporated rumen microorganisms, resulting in enhanced digestibility compared to the enzymatic method, which employed commercial enzymes. Statistical analyses revealed substantial variability among the collected ecotypes. Heatmap analysis showed that ecotype (E1) showed the highest OMD and ME, testifying to possible higher greenhouse gas emissions such as methane, especially because digestibility was negatively correlated with condensed tannins, which are known to mitigate greenhouse gases. While *Sulla flexuosa* represents a promising forage resource for ruminants, the selection of ecotypes should balance nutritional quality with environmental sustainability, particularly in relation to their influence on ruminal fermentation and greenhouse gas emissions.

452 Evaluation of the diversity and nutritional quality forages through NIRS signatures captured in the field

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Characterization of genotypes conserved in genebanks is crucial to promote their use in breeding programs or to incorporate outstanding varieties directly into agri-food systems. Since the 1970s, the International Center for Tropical Agriculture (**CIAT**) forage collection has conserved approximately 22,000 tropical forage accessions representing 690 species. Ninety-three percent of the accessions belong to the Fabaceae family and 7% to the Poaceae family. Traditionally, genebank characterisations focus on capturing basic morphological and phenological traits to support genetic quality assessments and inform regeneration and seed production decisions. However, due to the high demand for skilled personnel, these characterizations often do not explore traits related to productivity, nutrition or environmental impact. High-throughput phenotyping is becoming increasingly robust with more accurate sensors and models to assess productivity traits. For example, in the case of forages the analysis of reflectance observed in both the visible and near-infrared (**NIR**) wavelengths is widely used to quantify forage nutritional quality, significantly reducing the costs of nutritional assessments, traditionally expensive in laboratory settings. In this study, 300 forage accessions sown in the first half of 2024 are being evaluated under the environmental conditions of Palmira, at CIAT campus. These accessions are legume species adapted to tropical lowland conditions, characterised by their tolerance to low soil fertility and high aluminium levels, common challenges in many tropical regions. Hyperspectral signatures have been captured using the ASD QualitySpec, a portable spectrometer that capture measurements in the range of 350 to 2500 nanometres. These sampling is made when the plants reach at least 20% flowering. In the first half of 2025, spectral data are being analysed under different traditional and artificial intelligence models based on linear regression, generalised linear models, support vector machine and random forest, used to identify or predict the spectral regions that contribute most to the nutritional variability of the samples, also studying the correlation between spectral signatures and the mitigation of methane emissions measured under in vitro conditions, based on known regions of the visible and NIR spectrum associated with anti-methanogenic compounds such as tannins, flavonoids and saponins. Models are also being used to assess the functional diversity of accessions, applying hierarchical clustering to reveal similarities between accessions based on key spectral regions. It is expected that by mid-2025 conclusive results will be available on which model can establish these functional relationships, which enhance the assessment of environmental benefits, and which can identify the potential of accessions as a promising forage. The importance of this method lies in its ability to identify nutritional attributes and anti-methanogenic properties directly in the field with reliable validations helping to understand the conserved germplasm in order to recommend its use in a scientifically manner. These advances contribute to the selection of promising forages, foster the development of sustainable production systems in tropical regions and support climate resilient livestock farming under difficult conditions, while reducing greenhouse gas emissions. Also, It could be a method that is particularly advantageous in remote regions lacking access to quality animal nutrition laboratories.

453 Effects of bioprotect and brown sorghum tannin on rumen degradation, digestibility, and amino acid profile

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Condensed tannin can bind with crude protein in the rumen and form a tannin-protein complex, making the feed unavailable for bacterial colonisation and rumen fermentation. This experiment aimed to evaluate the effect of two tannin extract (**TE**) (20 and 40 g/kg dry matter (**DM**)) and two Bioprotect (15 and 30 mL/kg DM) inclusion rates compared with tannic acid (30 g/kg DM) on *in vitro* rumen protein degradation, *in vitro* intestinal digestibility and the amino acid profile of soybean and canola meals. The gas production and ammonia-N concentration from untreated meals were greater than that of treated counterparts ($P < 0.001$). The rumen degradable protein (**RDP**) of the untreated canola meal was greater than that of treated meals ($P < 0.001$), and it was not different between meals treated with 30 mL/kg Bioprotect and 40 g/kg TE DM. All additives increased the rumen undegraded protein (**RUP**) of both meals ($P < 0.001$), and no difference was observed between the proportion of RUP measured from canola and soybean meals treated with 30 mL/kg Bioprotect, 40 g/kg TE DM or tannic acid. The *in vitro* intestinal digestibility of RUP (**IVIDP**) was greater for canola meal treated with 40 g/kg TE DM and 30 mL/kg DM Bioprotect, followed by tannic acid ($P < 0.001$). Treating canola and soybean meals with additives increased the total essential amino acids (**EAA**) concentration quantified after *in vitro* rumen fermentation and IVIDP procedures. The use of 40 g/kg TE and 30 mL/kg Bioprotect DM resulted in greater total EAA than other treatments ($P < 0.001$). The reduction in gas production, ammonia-N and RDP, and the increase in RUP, IVIDP, and total EAA confirms successful protein protection in this experiment. Moreover, the use of 30 mL/kg Bioprotect and 40 g/kg TE DM were comparable with the positive control, tannic acid, in terms of most parameters tested in this experiment. However, *in vivo* studies are required to validate these findings on ruminants.

456 Mitigating methane emissions in livestock: evaluating grass-legume silage combinations

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Ruminants are a significant source of methane emissions in modern agriculture, contributing around one-third of global anthropogenic methane emissions. To address this, innovative feeding strategies are needed to reduce methane emissions without negatively impacting livestock productivity. This study investigated the proximate composition and methane production of silages made from various grass-legume blends. The silages were produced using freshly harvested *Panicum maximum* and *Centrosema molle*, which were wilted and chopped before being ensiled in 6.5-liter plastic laboratory silos using different ratios: 100% grass, 75% grass-25% legume, 50% grass-50% legume, and 25% grass-75% legume. The silages were incubated for 21 days and analysed in triplicate for proximate composition and methane production using in vitro methods. The data were then analysed using one-way analysis of variance to determine the effects of the different grass-legume combinations. The results showed that 100% grass silage had the highest ($P<0.05$) dry matter and ash content, while the 25% grass-75% legume silage had the highest ($P<0.05$) crude protein content. In contrast, the mixed silages had higher ($P<0.05$) methane production (9.33, 10.00 and 12.00 ml/200mg), organic matter digestibility, and short-chain fatty acid production compared to the 100% grass silage. The 100% grass silage exhibited the highest ($P<0.05$) in vitro dry matter digestibility (27.63mmol), significantly surpassing the other treatment groups. Therefore, incorporating mixed grass-legume silages (specifically 75% grass-25% legume, 50% grass-50% legume, or 25% grass-75% legume) into livestock feeding strategies may potentially reduce methane emissions while maintaining or improving nutritional quality.

458 Modelling greenhouse gas emissions from local dairys / effect on feed types on milk quality, Cameroon

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Promoting sustainable animal production is crucial in a developing country like Cameroon, where key elements of sustainability like, mitigation/adaptation and production are still at the developmental stage. The use of non-renewable sources of energy in industries to run mechanical systems and automobiles were said to be one of the visible explanations of the emission of GHG but research has shown that a significant amount of the earth surface is occupied by agricultural activities that in tend play a significant role in the emission of GHG. Most of the agricultural emissions arise from the livestock sector released during animal production and use of livestock manure. Agriculture is said to be a source and sink of GHG: A source because it is a net contributor of greenhouse gases to the atmosphere and a sink since it withdraws GHG from the atmosphere. This therefore conveys the necessity to know and understand the science behind animal production while ensuring a safe, clean and affordable agricultural emission mitigation approaches. Such conservation practices to prevent erosion, managing nitrogen to match crop needs and manipulation of animal diets amongst other mitigation practices have proven to drastically attenuate the emissions of carbon dioxide, methane and nitrous oxide. Livestock products constitute a reasonable amount of the human diet and it is a prerequisite to produce healthy products at affordable costs, but increasing milk yield and its resilience in our community as well as managing the drastic increase in GHG emissions from enteric fermentation most especially, are two major threats facing the dairy sector in Cameroon. This research, therefore, aims at analyzing the contributing effect of different feed types to CH₄, N₂O and CO₂ emissions in some dairy farms using local breeds (White Fulani and Gudali), as well as analysing their effects on milk characteristics over a period of 12 months in the agro-ecological zones 2 and 3, Cameroon. We quantified and compared GHG emissions of bovine milk production systems in 02 AEZs being 75% of Cameroon's bovine milk production. Results show drastic increase in GHG emissions from enteric fermentation, the feeding management mitigation approach is the most developed and it's paramount to decrease enteric methane emissions from ruminants without altering animal production while improving feed conversion efficiency. Also, results showed GHG emissions at the farm level are lower in developed than in developing countries. Cameroon Ministry of Livestock aim to reduce milk importation by encouraging domestic production (Business in Cameroon, 2016). Also, cattle herding possesses several challenges, for instance over grazing which gradually accounts for the replacement of pasture by weeds which subsequently leads to increase in GHG emissions from cattle wastes. Level of Crude Protein fed in concentrate on milk production and N-efficiency reveals that despite lower level of grass production under local management, the difference in milk production between local and strip-grazing was non-significant. The production of GHG from ruminants and impact on climate changes are major concerns and decreasing enteric CH₄ emissions from ruminants without altering production is desirable to reduce GHG emissions/improve feed conversion efficiency.

462 Evaluation of a CRISPR/Cas9 based gene editing system for *Urochloa humidicola* (Poaceae)

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The agricultural sector is a major contributor to greenhouse gas emissions, with livestock being a significant source of methane (**CH₄**) through enteric fermentation. As forage quality, digestibility, and composition directly influence CH₄ production in the rumen, the CRISPR/Cas9 technology offers a promising solution to tackle this problem by targeting these traits. Due to its agronomic and economic importance, the forage grass *Urochloa humidicola* has been selected for the development of a gene editing protocol using *Agrobacterium*-mediated transformation of embryogenic calli. The **PDS** (*Phytoene Desaturase*) gene has been selected as a proof of concept. To date, single guide RNAs (**sgRNAs**) have been designed and tested in vitro. The deactivation of this gene is expected to produce albino plants that will be easily identified in vitro. Simultaneously, two genes, *Bmr6* and *FatB*, have been identified as candidates for improving digestibility and increasing production of anti-methanogenic compounds (**AMCs**) in this species. The *Bmr6* gene encodes the enzyme cinnamyl alcohol dehydrogenase (**CAD**), which plays a critical role in the lignin biosynthesis pathway. Evidence shows that mutation of *Bmr6* in sorghum results in reduced lignin content and increased cell wall digestibility, making fibers more accessible for livestock digestion. This leads to shorter fermentation times in the rumen, thereby reducing methane production, while improving feed intake and livestock productivity. In turn, the *FatB* gene encodes an acyl-ACP thioesterase involved in fatty acid biosynthesis. Silencing the *FatB* gene to reduce levels of saturated fatty acids can increase the availability of unsaturated fatty acids, altering ruminal fermentation pathways. This adjustment can create competition for hydrogen within the rumen, limiting the substrate available to methanogenic archaea, which use hydrogen to produce methane. Both genes have been identified in *U. humidicola*, and sgRNAs have been designed. These genetic modifications could significantly reduce methane emissions while enhancing the nutritional quality of animal products. As of now, all gene editing efforts and advances are also being supported by ongoing work to optimize *Agrobacterium*-mediated genetic transformation and refine regeneration protocols based on concept proof, ensuring the future successful implementation of the CRISPR/Cas9 technology in *Urochloa humidicola*.

468 Effect of physical activities on methane emission, parameter estimations and predictions in dairy cattle

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Methane emission is a complex trait and its recording in farm animals is still a challenge. Different technologies are proposed to measure methane (**CH₄**) emissions in dairy cattle including the handheld laser methane detectors (**LMD**). Over 9780 point-measurements of methane emissions with minimum duration of 3 minutes from 920 cows in 28 small-holders dairy farms in Ethiopia were recorded using LMD. Moreover, milk yield and the milk mid-infrared (**MIR**) profiles of cows were also obtained. Recordings were obtained on cows during different activities including feeding, ruminating, sleeping, and standing idle with 1791, 5322, 434, and 2233 records respectively. Overall average CH₄ production was 379 g/day. Methane production in feeding status was highest with 554 g/day on average. Average CH₄ production for ruminating, sleeping, and standing idle, were 331, 326, and 366 g/day respectively. We performed a partial least square prediction using milk mid-infrared profiles and prediction accuracy were 0.41, 0.26, 0.26, and 0.25 for feeding, ruminating, sleeping, and standing idle respectively. Feeding status not only had highest CH₄ production average but also highest prediction accuracy. Inclusive prediction with all other cow activities in addition to feeding decreased the overall prediction accuracy to 0.28. In addition, genetic parameter estimation using pedigree data with effect of type of cow activity as fixed effects in the model were performed. Estimated heritability were 0.09 and 0.12 and phenotypic variances were 43582 and 48462 g/day with and without cow activities in the models, respectively. Results show that animal activities play an important role not only in terms of measuring levels of CH₄ produced but also when training models to use proxies such as MIR data to predict methane emissions. Moreover, inclusion of cow activities accounted for much variation in parameter estimation. These findings may be more important in using technologies that measure CH₄ emission only during feeding in dairy cattle such as Greenfeed system and Sniffers. Since these technologies collect point measurements during feeding and generalise them to calculate total CH₄ production per day, there might be the need to investigate a correction factor to avoid overestimation of CH₄ production by these tools.

469 Early-life supplementation of specific *Lacticaseibacillus rhamnosus* strains reduces enteric methane emissions in growing dairy heifers

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Methane (CH₄) is the world's second most abundant greenhouse gas, with 27% of anthropogenic CH₄ emissions resulting from enteric fermentation in ruminant livestock. Lactic acid bacteria, including *Lacticaseibacillus rhamnosus* strains, are natural inhabitants of the mammalian gastrointestinal tract and have been used in livestock as direct-fed microbial products. However, their ability to influence CH₄ emissions from ruminant animals is poorly understood. The aim of this study was to understand the effects of specific *L. rhamnosus* strains on CH₄ emissions in growing dairy heifers managed on a pasture-based system.

L. rhamnosus FNZ118 (Kowbucha™ FNZ118) and *L. rhamnosus* FNZ142 (Kowbucha™ FNZ142) strains (5 x 10¹⁰ colony forming units/day in 3 g of maltodextrin) were fed daily to 20 New Zealand -Friesian heifer calves per treatment from birth to 14 weeks. Control calves (n=20) received a daily dose of maltodextrin (3 g/day) from birth to 14 weeks of age. The calves were weaned at approximately 14 weeks and grazed on pasture until 12 months of age. Methane emissions and dry matter intake were measured in respiration chambers at 6 weeks, 14 weeks, 9 months and 12 months of age.

Methane production was on average 20% lower (P>0.001) in the calves treated with *L. rhamnosus* strains, compared to the control group at all time points studied. Dry matter intake was on average 20% lower (P>0.001) in the calves dosed with *L. rhamnosus* strains at the time points studied, while liveweight was not affected. This suggests an improvement in feed conversion efficiency has been induced in the calves receiving these *L. rhamnosus* strains.

These findings demonstrate that early life provision of specific *L. rhamnosus* strains lower CH₄ production while improving feed conversion efficiency and have potential to mitigate enteric CH₄ gas emissions from growing dairy cattle. Early life treatment may be more applicable to pasture dairy systems where daily feed additives are harder to apply.

474 Effect of a high nonforage-NDF concentrate diet on growing steers and air emissions.

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The optimal concentration of forage-NDF diet in feedlots is not well defined. This study compares a high-energy, low forage-NDF feedlot diet for growing Holstein steers with an alternative single-pellet diet high in nonforage-NDF (Afiber®) supplemented with tannins (Silvafeed ByPro: 0.15% DM) and rumen buffers (1.0% DM). The experiment was a cross-over design with two treatments (Control and Afiber®), two periods (26-d adaptation and 5-d evaluation), and eight Holstein steers (four/treatment), housed individually. Diets were formulated to be isonitrogenous. The chemical composition of Control and Afiber® diets were DM: 91.4% and 92.0%; CP: 13.4 and 13.3%; NDF: 16.6% and 39.3%; ADF: 8.3% and 22.8%; LDA: 1.7% and 4.6%; and EE: 4.3% and 7.9%; ash: 3.4% and 2.8%; and nonfiber carbohydrates (**NFC**) 62.3 and 36.8%. Daily DMI offer and refusal, weekly body weight gain (**BWG**) and CH₄ emissions were recorded. Methane was estimated with the sulfur hexafluoride (**SF6**) tracer technique. Non-significant differences were observed in animals' DMI, feed efficiency, and BWG, which averaged 5.93 kg DMI/day, 0.20 kg BWG/kg DMI, and 1.21 kg BWG/day. Afiber® treatment reduced methane emissions compared to Control: total emissions, from 151 to 125 g CH₄/animal/day (P<0.02); intensity by 20%, from 130 to 104 g CH₄/kg BWG (P<0.01). A trend to reduced emission factors was observed for the Afiber® diet from 25.6 to 21.5 g CH₄/kg DMI (P<0.06). The Afiber® treatment reduced methane emissions without compromising DMI or BWG.

475 Tannin extract supplementation improves milk yield and reduces enteric methane in early-lactation dairy cows

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Enteric methane (**CH₄**) emissions from ruminants contribute significantly to GHG and represent a loss of dietary energy. Reducing CH₄ emissions while maintaining productivity is a key challenge in sustainable dairy farming. Tannins have demonstrated potential to modulate rumen fermentation, suppress protozoa, and improve nitrogen utilization. However, their combined effects on dairy cattle productivity, health, and environmental emissions require further investigation. This study aimed to evaluate the effects of dietary supplementation with quebracho and chestnut tannin extracts on milk production, rumen fermentation, blood biochemistry, rumen protozoal composition, and CH₄ emissions in dairy cows. Twenty early-lactation Holstein cows (50 ± 25 days in milk; average milk yield 38.5 ± 11.1 kg/day) were randomly assigned to two groups: a control group (**CON**; n = 10) receiving basal diet (**BD**), and treatment group (**TAN**; n = 10) receiving the same BD supplemented with 18.0 g/cow/day of quebracho (*Schinopsis lorentzii*) and 18.0 g/cow/day of chestnut (*Castanea sativa*) tannin extracts (silvafeed ByPro). The trial lasted 20 days and was conducted under commercial farm conditions in Czech Republic. The BD was formulated on a DM basis and consisted of grass-clover silage (15.1%), corn silage (25.2%), pea silage (16.8%), meadow hay (1.7%), rapeseed meal (3.4%), a protein concentrate comprising soybean meal and malt flour (11.8%), ground wheat (37.3%), brewers' grains (16.8%), and ground corn (5.0%). Cows were milked twice daily (at 05:00 and 17:00) and had ad libitum access to the BD, fresh water, and mineral licks. Individual milk yield was recorded daily throughout the experimental period. Individual samples of rumen fluid, milk, and blood were collected from each cow on the designated sampling days (day 10 and day 20). Methane emissions were measured individually for all cows on day 20 using a Laser Methane Detector Mini (Pergam, Italy) during two 5-minute observation periods conducted at 4 and 8 hours after feeding, with CH₄ concentrations recorded every 0.5 seconds. Tannin supplementation significantly increased ($P < 0.001$) milk yield, fat-corrected milk, and energy-corrected milk without affecting milk composition (fat, protein, lactose, casein, somatic cell count, or beta-hydroxybutyrate). Although blood urea nitrogen, milk urea nitrogen, non-esterified fatty acids, and total antioxidant capacity were significantly elevated in the TAN group ($P < 0.05$), all values remained within physiological ranges, indicating no adverse metabolic response to tannin supplementation. Rumen fermentation patterns were altered, with elevated levels of propionate, butyrate, isovalerate, A:P ratio ($P < 0.001$), and reduced ammonia concentrations ($P < 0.05$). Total protozoa decreased ($P < 0.05$), with Entodiniomorphida – a key H₂-producer – showing a significant decline ($P < 0.001$). Moreover, emissions were significantly lower ($P = 0.013$) in the TAN group compared to the control (132.2 vs. 153.3 ppm·m). Combined supplementation with quebracho and chestnut tannins improved milk yield, antioxidant status, and nitrogen metabolism, while positively modulating rumen fermentation and protozoal structure. The significant reduction in Entodiniomorphida suggests a microbial mechanism contributing to reduced methanogenesis. These findings support the use of tannins as a promising nutritional strategy for improving dairy productivity and reducing the environmental impact of ruminant livestock.

476 Effect of decreasing doses of nitrate on methane emissions in lactating dairy cows.

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Nitrate (SilvAirTM, Cargill Inc.) supplementation has been extensively evaluated as a dietary strategy to reduce methane production in ruminants. A recent meta-analysis on nitrate supplementation in lactating dairy cows fed TMR diets, showed an overall reduction in methane production of 20% when SilvAirTM was fed at 1.6% of the diet DM (equal to 1% nitrate), with no effect on production performance and similar reductions at higher doses. However, the impact of doses below 1% of nitrate supplementation on methane reduction remain unclear and warrants further investigation. Therefore, the objective of this experiment was to evaluate the effects of three levels of SilvAirTM supplementation on methane production and performance in lactating dairy cows. Twelve Holstein cows (averaging 37 kg/d in milk and 173 DIM) were used in a 3 x 3 Latin square with 3 periods of 28 days (21 d adaptation and 7 d data collection). Cows were fed a corn silage and alfalfa haylage based TMR (50.5% DM, 16.5% CP, 30.9% NDF, 23.0% starch, and 2.7% fat). Treatments were: 0% (**CON**), 0.5% (**LOW**), and 1% (**HIGH**) of DM as nitrate. Urea and CaCO₃ were added to the CON and LOW treatments accounting for the N and Ca in SilvAirTM. Cows were housed in a tie-stall barn, fed and milked twice daily. Gas production was measured using the GreenFeed system (C-Lock Inc). Each cow was brought to GreenFeed eight times over three days. The visits were spaced nine hours apart, resulting in eight samples per cow, distributed every three hours throughout the day. Data were analyzed with the lmer package in R by using a model that contained the fixed effect of diet and the random effects of period and experimental unit. For repeated measurements throughout the day, treatment, time and their interaction were also included in the model. HIGH reduced methane production by 17.8%, and LOW by 13.7% compared with CON (quadratic effect $P = 0.08$). There was no treatment by time interactions in the gas measurements. Supplementation of nitrate linearly decreased ($P = 0.04$) DMI compared with CON (-0.5 and -1.2 kg/d for LOW and HIGH, respectively) but did not affect ($P > 0.25$) milk production (~33.7, 34.5, 33.5 kg/d $\pm$ 1.7 for CON, LOW and HIGH respectively) or ECM yield (39.0, 39.5, 38.2 $\pm$ 2.0 kg/d for CON, LOW, and HIGH respectively). Methane yield (15.7, 13.7, 13.7 $\pm$ 1.6 g/kg DMI for CON, LOW and High respectively) and intensity (10.9, 9.2, 9.2 $\pm$ 1.0 g/kg ECM for CON, LOW and HIGH respectively) reduced ($P = 0.02$ and $P = 0.01$ respectively) quadratically as nitrate dose increased, but the effects were similar between LOW and HIGH. This study shows that supplementing a nitrate dose of 0.5% of DMI can significantly reduce methane production and yield in dairy cows without negative effects in production, suggesting that more research is needed to find the optimal dose.

477 Effect of nitrate on enteric methane in beef cattle fed a concentrate and straw-based diet

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This study aimed to evaluate the impact of supplementing calcium nitrate (SilvAir®, Cargill Inc.) on enteric methane emissions in beef cattle fed a concentrate and straw-based diet. The trial was carried out at a commercial feedlot in Valderejo, Álava, Spain, involving 40 heifers of various breeds, including Limousin, Pyrenean, and crossbreeds. The animals were divided into two homogeneous groups: a control group and a treatment group that received a concentrate supplemented with 1.25% SilvAir®.

At the beginning of the trial, the animals had an average age of 8.4 months and an average body weight of 240 kg. The experimental period lasted six months, during which methane emissions were measured using the GreenFeed system (C-Lock, Inc). The system recorded emissions during alternating months for each group to ensure balanced data collection. Body weight was measured at the beginning and end of the trial, so average daily gain (ADG) could be calculated. Dry matter intake was monitored to assess the impact of the additive on animal performance. Concentrate intake was estimated for the whole feeding period as the difference between the quantities available at the start of the trial offered andorts at the end for each group. The results showed no significant differences in final body weight (439 vs 416 kg, $P=0.19$) and ADG (0.95 vs 1.07 kg/d, $P=0.14$) between the control and SilvAir® groups, indicating that calcium nitrate did not negatively affect growth performance. A significant reduction in methane emissions was observed in the SilvAir® group. Specifically, methane production decreased by 36.8% (from 158 g/day in the control group to 99.8 g/day in the treatment group; $P<0.001$). Methane yield tended to decrease by 20.4% (from 152 g/kg ADG in the control group to 121 g/kg ADG in the treatment group; $P=0.058$).

This reduction exceeded the expected stoichiometric potential of nitrate as a hydrogen sink, which typically predicts a 25.8 g CH₄ reduction per 100 g of nitrate. In this study, 94 g of SilvAir® intake led to a 58.2 g/day reduction in methane, implying a higher-than-anticipated efficiency. This could be due to additional antimicrobial effects of nitrate or nitrite on methanogenic archaea or hydrogen-producing bacteria, beyond the stoichiometric hydrogen capture. Previous studies have also reported similar antimicrobial effects and shifts in methanogen populations. Unlike most previous studies that used high-forage diets, this trial used a high-concentrate diet, which may have influenced the observed outcomes.

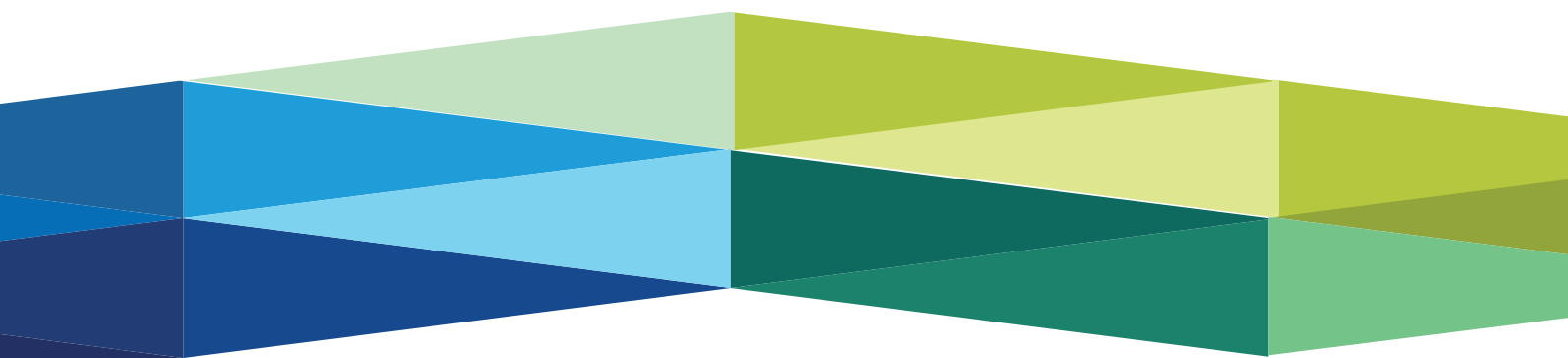
In conclusion, the inclusion of SilvAir® in the concentrate of heifers fattened on a concentrate-straw diet significantly reduces enteric methane emissions without compromising animal performance. These results support the use of dietary nitrate as a viable methane mitigation strategy in beef production systems, particularly under high-concentrate feeding regimes.



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Theme 2: Animal health, genetics, and genomics for climate resilience



22 Decoding pre-weaning heat stress effects and gene networks in neonatal dairy calves for climate-resilient breeding

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Pre-weaning heat stress presents a significant challenge in livestock production, adversely affecting growth, health, and overall productivity in dairy calves. This study aimed to elucidate the molecular and genetic mechanisms underlying calf responses to heat stress, with a particular emphasis on gene expression profiling. The research utilized the RNA-Seq dataset GSE227323 from the NCBI Gene Expression Omnibus (GEO) to investigate the effects of pre-weaning heat stress on Holstein dairy calves. The dataset comprises RNA-sequencing data from liver tissues collected at different postnatal stages, enabling a comprehensive assessment of gene expression changes in response to thermal stress. Calves were subjected to either postnatal heat stress (HS), characterized by sustained exposure to ambient temperatures exceeding 32°C with relative humidity above 60%, or thermoneutral (TN) conditions, maintained between 20-22°C for optimal physiological function and development. Comparative transcriptomic analysis identified several differentially expressed genes (DEGs) associated with heat stress adaptation, oxidative stress response, and metabolic regulation. Key upregulated genes in HS-exposed calves included HSP90AA1, HSPA1A, and HSF1, which play crucial roles in cellular stress protection and protein homeostasis. Downregulated genes such as IGF1, PCK1, and G6PC indicated disruptions in metabolic pathways, particularly those related to growth and energy metabolism. Functional enrichment analysis of significantly upregulated genes revealed key biological processes and pathways, including keratinocyte regulation and skin barrier maintenance, which are critical for the health of heat-stressed animals. The integration of transcriptomic data with pathway analysis serves as an approach to unraveling complex molecular responses in the face of environmental stressors. These findings are suitable for enhancing the understanding of how pre-weaning heat stress impacts calf physiology, with potential of laying the foundation for targeted interventions such as prenatal maternal nutritional supplementation aimed at pre-calving programming of calves to improve stress resilience and growth performance. Additionally, these insights can also contribute to development of effective mitigation strategies to improve animal welfare and productivity in increasingly warm climates, highlighting the necessity of genetic and management interventions in sustainable livestock production. Given the increasing impact of climate change on livestock systems, the understanding of how heat stress influences metabolic and physiological functions in calves is crucial for developing greenhouse gas mitigation strategies. By identifying key genetic and molecular pathways linked to thermal adaptation, this study has potential of advancing research on the mitigation of climate-induced stressors in animal production. The study findings provide insights that can inform policies and farm practices aimed at reducing the environmental footprint of livestock while ensuring calve productivity and animal welfare.

33 Animal Health, Genetics and Genomics for Climate Resilience

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Livestock contribute to about 25% of total agricultural GDP in low and middle income countries. Meanwhile, livestock production is under threat because of climate change. Rising temperatures and unpredictable weather patterns have an impact on how livestock feed, produce and reproduce.

Livestock movements during periods of drought causes temporary concentration of animals around watering points and pasture lands, resulting to land degradation and water scarcity. Livestock movements spread livestock diseases and pests. Livestock disease outbreak and pests infestation occur during changes of climatic conditions. Foot and mouth disease is common during dry seasons while rift valley fever occurs during flooding seasons in areas with high temperatures (ASAL's). Arid and semi-arid counties in Kenya under ASAL are the following. Arid Counties: Baringo, Garissa, Isiolo, Mandela, Marsabit, Samburu, Tana River, Turkana and Wajir. Semi-arid counties: Embu, Kajiado, Kilifi, Kwale, Laikipia, Lamu, Makueni, Meru, Narok, Nyeri, Taita Taveta, Tharaka North and West Pokot. Pests such as ticks thrive in areas where high temperatures alternate with wet conditions.

Effects of climate change on livestock: Loss of animal biomass, high mortality, disruptions of normal livelihoods of livestock owners, dependence on external relief and massive floods. Drought causes competition between livestock and wildlife resources. Lack of vegetation cover causes death to animals and loss of income to the affected communities. The occurrence of droughts and floods are common in ASAL counties with a cyclic pattern occurring every 5 to 7 years. Global warming has resulted to very little time to recover before the next drought sets in. ASAL's are fragile ecosystems that require careful intervention to prevent environmental degradation and desertification. The community also participates in identifying, planning, implementing and evaluating development projects in order to achieve sustainable. Early warning systems are critical to reduce the vulnerability to natural disasters such as droughts. Resilience is the ability to maintain functioning in the face of adversity, it is influenced by both environmental and genetic factors. Genetic resilience provides resilience to disease onset or progression. It therefore leads to a higher survival rate and therefore increased livestock production.

Using genomic tools, scientists can assess a population's ability to adapt to their changing environment and predict whether it will be vulnerable to climate change. Genomic tools can be used to increase animal resilience, improve disease resistance, reduce methane emissions from cattle and sheep. Genomic testing in cattle allows early prediction of genetic merit, it increases the value of genetic merit and it increases the value of young breeding stock. Genomic selection allows cattle breeding in developing countries to intensify their production and adapt to the changing environment.

47 Animal Health, genetics, and genomics for climate resilience

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Climate change poses significant challenges to animal health and agricultural systems, necessitating innovative approaches to safeguard livestock and wildlife. This paper explores the integration of animal health management, genetics, and genomics to enhance climate resilience. With rising global temperatures, shifting precipitation patterns, and the emergence of novel pathogens, the vulnerability of animal populations has escalated. Addressing these challenges requires a multidisciplinary approach, combining traditional veterinary practices with advancements in molecular biology.

The paper highlights the critical role of genetics and genomics in identifying and propagating traits associated with climate adaptability, such as heat tolerance, disease resistance, and improved metabolic efficiency.

54 Climate-smart agriculture in poultry farming in Southeast Nigeria

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In climate change mitigation, efforts are made to reduce the impact of climate change by decreasing greenhouse gas emissions. Climate-smart agriculture in poultry farming involves the use of innovative technologies and strategies that improve resilience to climate change, reduce greenhouse gas emissions and ensures sustainability in agriculture. Application of these strategies will enhance poultry farming systems' ability in the reduction of climate-related stresses. This study aimed to evaluate the climate-smart agriculture practices used by poultry farmers in Southeast, Nigeria. Specifically, the study describes the socioeconomic characteristics of the farmers, determine the frequency of use of climate-smart agriculture and to ascertain the perceived benefits of climate-smart Agriculture. A total of 240 poultry farmers' responses were collected with the use of a structured questionnaire. Multistage sampling technique was used to select the 240 poultry farmers used for the study. Frequency, percentages and mean were the statistical tools used to analyze the socioeconomic characteristics of the farmers, frequency of use of the climate-smart agriculture practices and the perceived benefits of climate-smart agriculture. Hypothesized relationship between the socioeconomic characteristics and the use of climate-smart agriculture were established using multiple regression analysis. The result of the study revealed that there were more female farmers (52.5%) while (47.5%) were males in the sample used for the study. The respondents were mostly adults with a mean age of 38 years, 22.5 % had secondary school while 47.5% had their tertiary education. The farmers perceived among other benefits that climate-smart agriculture enhanced resilience to climate change with the mean score of 2.87 as the highest mean score while the other benefits listed have mean scores of above cut-off mark of 2.5 under four rating scale of agreement. The results of the multiple regression analysis refuted the hypothesis of a non-significant relationship between socioeconomic characteristics of the respondents and the use of climate-smart agriculture. The result of the multiple regression revealed that 63% of the variation in the use of climate-smart agriculture could be attributed to some of these socioeconomic characteristics. Education, occupation and income were statistically significant at 5% probability level. In conclusion, the study provided valuable insights into the potential transformative impact of climate-smart agriculture in ensuring environmentally friendly food system in poultry farming as evidenced in the perceived benefits recorded. Also education plays important role in the promotion of the use and optimization of climate-smart agriculture in the study area.

79 Estimated methane efficiency and expression of breed and heterosis effects of different beef cattle genotypes

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The concept of sustainable development is increasingly important in the agri-food sector and global economy. Food security represents an increasing challenge, both locally and globally, coupled with the need to reduce the carbon footprint of livestock production. Emissions from global livestock account for up to 14.5% of anthropogenic greenhouse gases emissions and up to 44% of livestock emissions are in the form of methane. There are several alternatives to decrease methane emissions, such as improving feed quality, using methane inhibitors, as well as the utilization of breeding for lower methane emissions. In this study, the methane efficiency (kg) was estimated as residual 205-day weight from analysis of covariance in which the Large Stock Unit of each animal served as the covariate. Because a Large Stock Unit has been shown to produce 94 kg of enteric methane per year in South Africa this analysis is equivalent to one in which the breed group means were adjusted to a constant amount of methane produced. The residual weaning weight is the amount by which the adjusted weight deviates from its expected value. No division is involved in calculating the methane efficiency values. Where the division comes in to play is in calculating the relative efficiency values. The relative methane efficiency (%) was estimated as breed group mean methane efficiency divided by the mean 205-day weight (189.02). Breeds of dam are not significantly different from each other. The Angus breed of sire is significantly more efficient relative to methane produced from the Afrikaner, Nguni and Simmentaler, but not different from the Bonsmara. Estimated heterosis was 4.47 ± 3.23 ($P = 0.17$). The highest methane efficiency of 114 was expressed by the Afrikaner sired by the Simmentaler cross, with a heterosis effect of up to 14%. Additionally, the Angus sired calves on average expressed methane efficiency of 105 with a heterosis effect of up to 5%. Note, these values of methane efficiency are philosophically consistent with feed efficiency being estimated as residual feed intake. Environments that impact various systems will be evaluated through calculation of their carbon footprint based on the feed energy that is required to support the system. Further research will make use of bioeconomic simulations to predict efficiencies for utilization of different breed resources.

88 Recording methane production phenotypes from composite and crossbreed beef cattle grazing tropical and subtropical pastures.

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Enteric fermentation in cattle produces considerable anthropogenic methane emissions, including in tropical regions, contributing to climate change and reducing production efficiency. Methane production is a heritable trait, however genomic selection to reduce emissions depends on collecting representative phenotypes. To generate these phenotypes, GreenFeed units (**GFU**) have been utilized under near-commercial conditions throughout Queensland to determine methane production of cattle grazing tropical and sub-tropical pastures. Additionally, factors contributing to variation in methane production from cattle utilizing short term breath measurements were investigated.

Cattle (n=760) were monitored in 7 trial groups across 4 locations in Queensland, Australia between July 2023 and July 2024. Four trials (**T1**, **T2**, **T4**, **T5**) were conducted across two Queensland Department of Primary Industries research sites, each comprising of 75 heifers and using 4 GFU. Emissions from 35 (46%), 38 (51%), 67 (89%) and 61 (81%) heifers were recorded, respectively. The other two trials were conducted on collaborating commercial properties. Trial 3 (**T3**) recorded 127 of 300 (42%) mixed sex cattle using 12 GFU. Trial 6 and 7 (**T6**, **T7**) used 4 GFU and 80 cattle per trial, 60 (75%) bulls and 65 (81%) heifers recorded, respectively. Average trial length was 38 days, and pre-trial cattle were familiarised with GFU for a period of 3 weeks. Factors were specified as nested fixed effects in a linear mixed model with cattle and test day fitted as random effects. *Bos taurus indicus* percentage, sex, rainfall, and temperature-humidity index (**THI**) were considered but were not retained due to lack of statistical significance.

Methane production was successfully estimated for 453 (of 760) trial cattle, resulting in an average trail visitation percentage of 64.7% (95% CL: 53.8, 75.6) across the 7 trials. Visitation typically reflected a diurnal pattern, with cattle showing increased attendance (34,192) during daylight hours (5:00-16:00), when compared to outside this window (12,703; $P < 0.001$). A moderate positive correlation (0.66, $P < 0.001$) was observed between methane (**CH₄**) production and carbon dioxide (**CO₂**) production. The largest variability in methane production was attributed to estimated total standing dry matter (**TSDM**), trial location and initial weight. Cattle showed a slight preference for GFU resulting in a small proportion of variation attributed to GFU (0.2%). Factors retained in the final model for estimated methane production included: daily visitation ($P < 0.001$), visit duration ($P < 0.001$), TSDM ($P < 0.001$), trial ($P < 0.001$), initial liveweight ($P < 0.001$), pipe temperature ($P < 0.001$), GFU ($P < 0.001$), trial visitation ($P < 0.001$), and hour of visit ($P = 0.03$). This study demonstrates the exciting potential of genomic selection to reduce methane emissions from tropical beef cattle by identifying lower-emitting individuals. Additionally, it demonstrated that high-quality phenotypes can be effectively generated using GFU at a moderate scale under near-commercial conditions on tropical pastures. Although minimal variation was observed between GFU, the results of this study suggest that incorporating key influencing factors, such as weather conditions, trial, TSDM, and initial liveweight, is likely to enhance the accuracy of methane production estimates.

93 Evaluation and implementation of the economic & sustainability boost from the slick gene in cattle

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A large proportion of the global cattle population lives under predominantly tropical conditions where poor efficiency and high methane density of meat and milk production has been highlighted by FAO and others. Cattlemen and researchers have been aware of the dominant SLICK gene effect in Senepol and Carora cattle for several decades, and the actual gene location was discovered by Livestock Improvement Corporation (**LIC**) in New Zealand in 2014. The effects of the gene on animal welfare and productivity, under true tropical conditions, have been quantified by several research teams, e.g. University of Florida in USA, plus Venezuela, Puerto Rico and recently Costa Rica and Philippines. The SLICK carrier cattle have shorter hair coat and more sweat glands in their skin, enabling them to dissipate internal heat more easily and keep their core body temperature up to 1 degree lower. This effect was recorded even in barns with fan cooling and misting (by University of Florida). Hence the cattle are less stressed and eat more normally both in housed and pasture grazing systems, but the net economic impact and a method to include the gene in genetic rankings has not been previously described. The impact of Heat Stress on cattle welfare and performance has been well described in the literature. Heat Stress impacts performance and feed conversion efficiency through:

- 1) Growth. Heat stress increases the maintenance energy requirement by 15%, and feed intake is reduced in cattle under Heat Stress. The combined effect can halve growth rates for Bos Taurus cattle in the tropics.
- 2) Lactation. "Cows exposed to heat stress experience a 15-25% reduction in milk yield," Hansen 2013, a combination of the effects of higher maintenance requirement and reduced feed intake
- 3) Fertility. "Reduced expression of symptoms of heat and lower fertility after insemination (as low as 10-20%)". Hansen 2013. Holstein cows with the SLICK gene were recorded to get back in calf on average 60 days earlier than their non-SLICK herd mates, which lose body condition faster and will be less active when under heat stress. The extended calving intervals and dry periods are costly and reduce lifetime calvings and days in milk. Less heifer calves are available for herd maintenance and improvement.

The compounding impacts of these effects reduce feed conversion efficiency, and delay farmer income to cause major economic and environmental damage. When the Heat Stress effect is mitigated in cattle with the SLICK gene, the improvement in feed conversion, (and water), efficiency and lifetime profitability is dramatic, in the order of US\$2,000 to \$9,000 per head depending on the seasonal duration of tropical conditions and local prices. This improved productivity is matched with reduced methane density per kilogram of milk produced, or of growth in younger animals. These trait effects can be readily calculated and combined to account for the gene effect in a customised Tropical Profit Index for sire selection fit for local conditions and will confirm that widespread uptake of SLICK genetics is a vital solution for tropical cattle sustainability.

117 Review on status of animal breeding for heat tolerance improvement in cattle

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The objective is to review the status of animal breeding for heat tolerance improvement in cattle. Economic losses due to heat stress have been significant. Heat stress occurs when THI greater than the animal's thermo-neutral zone. Many strategies were suggested and implemented to mitigate heat stress. Among them, breeding for improved heat tolerance could be taken as a cost effective and long term solution of the problem. Animal breeding have showed rapid and appreciable advancement. Nowadays, it has started shifting from quantitative based to molecular based breeding. Identification of heat tolerant cows could help to improve dairy performance through genetic selection. Several measures have been proposed as criteria to identify heat tolerant animals. Differences in thermal tolerance exist among cattle provide clues to select thermo-tolerant animals using genetic tools. The heritability of rectal temperatures under heat stress conditions in cattle ranged from 0.13 to 0.17. There is heat shock gene related to thermo-tolerance that was identified and being used as marker in marker assisted selection and genome-wide selection to develop thermo-tolerant breeding bull. Heat tolerance genomic breeding values were developed for Holstein and Jersey bulls with reliability of heat tolerance of 30%. Breeding strategies to improve heat tolerance depend on the production system. The genetic mechanisms of heat-stress sensitive and tolerance should be identified before trying to improve heat resistance in dairy cows and in order to optimize the animal breeding program, it is important to add molecular genetic techniques along with the conventional animal breeding methods.

131 Cost of reducing methane production through genetic selection in beef cattle

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Methane is a greenhouse gas produced by beef cattle as a by-product of rumen fermentation. When emitted into the atmosphere, it contributes to climate change. Genetic selection index is a tool by which beef producers can improve the economic merit of their cattle. Incorporating emitted methane into a selection index requires knowing the economic value of a unit increase in the amount of emitted gas. Currently, this economic value is unknown and cannot be estimated in a straightforward way. However, with an appropriate estimate of the additive genetic variance for emitted methane and its genetic covariances with the other traits in the selection index, the consequence of assigning various levels of relative economic value to emitted methane and its influence on total economic merit can be assessed using selection index methodology. A breeding objective was developed for a full-cycle production of Hereford cattle in the USA. Index coefficients (**b**) for estimated breeding values (**EBV**) for the weaning endpoint were found following Schneeberger et al. (<https://doi.org/10.1111/j.1439-0388.1992.tb00395.x>). The numerical index of EBV was: $I = 0.06$ (mature weight) + 5.08 (stayability) + 0.47 (maternal weaning weight) + 0.86 (direct weaning weight). Daily methane production rate was added to the breeding objective and this index by allotting it predetermined proportions of the relative economic values. Estimates of the genetic variances and covariances were drawn from various literature and proprietary sources. The resulting G matrix was not positive definite and was made positive definite using the mbend package in R and the method of Jorjani et al. ([https://doi.org/10.3168/jds.S0022-0302\(03\)73646-7](https://doi.org/10.3168/jds.S0022-0302(03)73646-7)). Using the EBV as selection criteria and fixing selection intensity at 1.0, genetic responses (R) per generation were predicted using selection index methodology. When methane yield was ignored in the breeding objective, response in the index was US\$ 34.45 and methane yield was predicted to increase by 0.70 g methane/d. Assigning methane production rate 20% of the relative economic value resulted in predicted responses of US\$ 33.39 and -1.04 g/d, respectively. When methane was considered of equal relative importance to the production index the predicted responses were US\$ 23.19 and -4.68 g/d, respectively. Holding the predicted response in methane production rate to zero required that its relative economic value account of 9.3% of the breeding objective. These values, while subject to a degree of uncertainty due to potential variability in the estimates of (co)variance, highlight the importance of considering methane production in breeding objectives for beef cattle. The consideration of methane production in these breeding objectives will require an extensive set of robust genetic parameter estimates resulting from extensive phenotyping of a large number of animals for a wide variety of traits including daily methane production.

178 Predicting methane emissions in ewes

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Reducing methane emissions from ruminant livestock, which contribute roughly 30% of global methane emissions, is essential for achieving sustainable agriculture goals. Advances in methane measurement, such as portable accumulation chambers (**PACs**), enable large scale recording in sheep. However, predicting methane emissions remains challenging due to complex interactions with animal performance traits. This research aims to enhance prediction accuracy by examining these complex interactions.

Methane emissions measured using PACs were available for 1,932 ewes, yielding 3,802 measurements. A number of potential factors were considered for the prediction of ewe methane production (g/day) including: hours off feed (number of hours the ewe was off feed prior to measurement), PAC run number (group ewe was included in for each PAC measurement), ewe weight and weight change, body condition score (**BCS**) and BCS change, ewe breed, ewe parity, days since lambing, litter birth weight and litter weaning weight. Ewe weight and BCS change were calculated as the difference between weight and BCS measurements within ± 120 days of the PAC measurement.

For the prediction of methane production four fold cross-validation was performed whereby the dataset was stratified by ewe and randomly divided into four groups. Cross-validation was performed using a linear regression model with the analysis run four times but with methane data masked for one group at a time with the masked methane data predicted on the entire remaining dataset. Significant fixed effects included: hours off feed, breed, weight, parity, ewe weight change, days since lambing, and litter birth weight. The model achieved an average root mean square error (**RMSE**) of 5.89 ± 0.16 g/day, R^2 of 0.51 ± 0.04 and bias -0.0131 ± 0.147 g/day. Results showed that methane production increased by 0.115 ± 0.010 g/day ($P < 0.001$) for each additional kilogram of ewe weight. For each additional hour a ewe was kept off feed prior to measurement her methane emissions reduced by 2.68 ± 0.096 g/day ($P < 0.001$). Methane production also differed by breed ($P < 0.001$), with the lowest emissions recorded in Belclare ewes, intermediate levels in Texel ewes, and the highest emissions observed in Suffolk, Charollais, and New Zealand Texels, which did not differ significantly from each other.

A model including dry matter intake (**DMI**) as a predictor was also investigated, however, this analysis was based on a subset of the data, 894 measurements across four breeds (New Zealand Suffolk, Texel, New Zealand Texel, and Suffolk). This model achieved an R^2 of 0.338 and a residual standard error of 5.576 g/day, which was lower than the R^2 of the initial model. This difference is likely attributable to the smaller dataset available for the DMI model. This highlights the importance of larger datasets to better capture the relationship between DMI and methane production in future analysis.

These findings demonstrate that factors such as days since lambing, breed, ewe weight, and weight change can help in the prediction of methane emissions in ewes. These insights can enhance the accurate modelling of methane production and inform strategies for emissions reduction.

212 The relationship between methane output and body composition in post weaning lowland and hill lambs

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Understanding the factors influencing enteric methane (**CH₄**) emissions in ruminants is essential if methane reduction targets are to be achieved. Previous studies have examined how data relating to animal body composition affects CH₄ emissions, including the relationship between methane output and rumen physiology in lambs. Lambe et al. (2022) discussed how selection for increased lean meat yield would result in lambs with smaller reticulo-rumen volumes, potentially lowering CH₄ emissions. Identifying traits such as carcass composition, both pre- and post-slaughter, may enable estimation of methane breeding values without intensive CH₄ measurements. Performance differences, including carcass traits, are commonly observed between Ireland's main sheep production systems: semi-intensive lowland and extensive hill sheep (O'Brien et al., 2015). However, variation in methane emissions and potential predictor traits between these systems has not been explored. The objective of this study was to assess the relationship between methane output and body composition data derived using CT in lowland and hill lambs post-weaning.

The study took place at Teagasc Athenry, Co Galway across two years in September 2023 and 2024. Data from 820 lambs representing both lowland and hill breeds was obtained. All animals were on a similar diet prior to data collection. Enteric CH₄ output was measured using portable accumulation chambers (**PAC**) on all lambs prior to body composition data, including rumen volume, being collected using CT scanning. A total of 710 lambs were slaughtered within 9 days of measurement, resulting in the collection of slaughter and carcass data. On average, lowland lambs were approximately 185 days of age at CT scanning and weighed 43.03 ± 3.43 kg. Hill lambs were approximately 153 days of age at CT scanning and weighed an average of 29.48 ± 3.64 kg. The phenotypic relationship between methane emissions and body composition traits and lamb type (lowland versus hill) were investigated using Pearson correlations. Across all lambs, the average rumen volume was 5.99 ± 1.39 L, while CH₄ output averaged 15.57 ± 5.65 g/day, with hill lambs having lower rumen volumes (-1.77 L) and daily methane output (-5.14 g/day) in comparison to lowland lambs ($P < 0.001$). The weight of total muscle tissue was, on average, 11.11 ± 2.95 kg across all lambs, while fat tissue averaged 2.57 ± 0.61 kg. Across all lambs, a moderate positive phenotypic correlation (0.33 ; $P < 0.001$) was observed between CH₄ output and rumen volume and ranged from 0.12 in lowland to 0.25 in hill lambs. Moderate correlations were observed between the total weight of muscle tissue and CH₄ output (0.38 ; $P < 0.001$) and between CH₄ output and the total weight of fat tissue (0.22 ; $P < 0.001$), respectively. When adjusted for live weight of the animal the correlations between CH₄ output and muscle and fat tissue weakened and were closer to zero. From this study it is evident that there is a stronger relationship between the muscle tissue component of the body and CH₄ output when compared to rumen volume or the fat tissue component of body composition across all lamb types included in this study.

266 Does ewe genetic merit influence methane output? An investigation via a field based case study

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Globally ruminant production systems are striving to meet efficiency targets in order to optimise animal performance and economic viability. Pressure is mounting on agricultural producers to lower their farm carbon footprint (Rojas-Downing et al., 2017); of which methane (**CH₄**) is one of the main greenhouse gases being produced. Enteric CH₄, produced as a by-product of digestion in ruminant animals, is one of the largest contributors to global livestock emissions (Garnsworthy et al., 2019). Therefore it's production and mitigation has become a primary focus area. Studies have shown that methane output is heritable (Jonker et al., 2018; McHugh et al., 2022; Pagoni et al., 2017). Therefore the role of animal genetics in mitigating methane output is currently under investigation (Farrell et al., 2022). The objective of this study was to delve into the effect of ewe genetic merit on both performance traits and methane output using a field based case study. The study consisted of three genetic merit groups (New Zealand (**NZ**), elite genetic merit Irish (High Irish) and low genetic merit Irish (Low Irish) ewes and ran from 2020-2024, inclusive. Each genetic merit group contained 25 purebred Suffolk (**SU**) and 25 purebred Texel (**TX**) ewes, that were selected based on their maternal genetic indexes in their country of origin namely Ireland (Euro-star Replacement index) or New Zealand (New Zealand Maternal worth). Methane output was measured using Portable Accumulation Chambers (**PAC**) from ewes each year when animals were lactating and non-lactating. Ewe body condition score (**BCS**), ewe body weight (**BW**) and progeny performance was recorded. Dry matter intake (**DMI**) measurements were collected on a subgroup of ewes both when lactating and dry and all parameters were analysed using linear mixed models. Fixed effects included in the model were ewe genetic merit, breed, parity, time since feed removal and chamber number. Body weight was included as a covariate across groups and a contemporary group combining date and lot number of measurement was included as a random effect. The final dataset contained 1923 methane measurements from 539 animals. At the point of methane measurement ewe BW and BCS did not differ by genetic merit, however differences were observed between breeds with SU ewes being heavier and having a lower BCS than TX ewes ($P < 0.05$); regardless of country of origin. Methane output was 20% higher when recorded in lactating versus non-lactating animals ($P < 0.05$) while lactating ewes ate on average 500 g DM/day more than non-lactating ewes. Methane output across production stages differed by BW, BCS, parity, time since last feeding, litter weaning weight and genetic group ($P < 0.01$). High Irish ewes had a lower methane output than both Low Irish ewes and NZ ewes ($P < 0.05$), thus reflecting perhaps the stabilisation of BW within the overall index. In conclusion, animal genetic merit influenced methane output alongside further production traits of interest. This highlights the need to understand methane output in conjunction with key performance indicators and the importance of measuring methane to incorporate into genetic indexes; thus acting as a mitigation tool in sheep.

400 Host genetics and rumen microbiome impact on methane and feed efficiency traits in Hereford cattle

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Enteric methane emissions represent a loss of 6 to 12 % of gross energy intake, which impacts feed efficiency. The rumen microbiome plays a key role in this process. We aim to investigate the contributions of the host genome and rumen microbiome to methane emissions and efficiency traits.

We used a dataset of 537 Hereford bulls and steers, with dry matter intake (**DMI**) and residual feed Intake (**RFI**) information recorded in 70-day feed efficiency tests. A subset of 123 animals also had methane intensity (**MI**) and yield (**MY**) data based on methane (in g/d) records collected using GreenFeed during the same period. All animal's DNA was genotyped using medium-density panels (87,000 SNPs), and rumen microbiota was sequenced by enzyme-restriction reduced representation sequencing. Variance components of the animal genome, rumen microbiome, and the interaction effects were estimated for DMI, RFI, MY, and MI traits with four models: (1) model G, with the additive genomic effect only; (2) model M, with the microbiome effect only; (3) model GM, with the genomic and microbiome effects; and (4) model GMI, with the genomic, microbiome, and interaction effects. Analyses were performed using the Bayesian reproducing kernel Hilbert space regression method. The following parameters were computed for DMI, RFI, MY, and MI: 1) heritability (h^2) as the proportion of the phenotypic variance explained by the genetic effect (model G), 2) direct heritability (h^2_d) and microbiability (m^2), representing the decomposition into the direct genetic effect and the microbiome effects on the phenotype, respectively (models GM and GMI).

The h^2 for DMI, RFI, MY, and MI were 0.29 ± 0.07 , 0.22 ± 0.07 , 0.33 ± 0.14 and 0.45 ± 0.15 respectively. The h^2_d , calculated with model GM, were 0.22 ± 0.06 , 0.18 ± 0.06 , 0.22 ± 0.11 and 0.25 ± 0.12 , whilst the estimates were 0.18 ± 0.05 , 0.15 ± 0.06 , 0.15 ± 0.09 and 0.18 ± 0.10 when using the GMI model. The values of m^2 from the model containing only the rumen microbiome matrix were 0.27 ± 0.07 , 0.17 ± 0.06 , 0.22 ± 0.09 , 0.33 ± 0.12 for DMI, RFI, MY, and MI respectively. These estimates dropped by 25-55% as the complexity of the model increased when used in conjunction with the genomic and interaction effects. Our results showed that the magnitude of h^2_d was always lower than the h^2 in all traits, indicating that part of the variance explained by the phenotype derives from the microbiome component, which mediates the host genetic effects. The combined analysis of rumen microbiome and genomic information allows identifying the relative effects of the host genome that are mediated by the microbiome on methane and feed efficiency traits. These findings support further investigation of the role of rumen microbiome composition in selection programs.

404 Leveraging digital innovation in livestock breeding practices to lower methane emissions

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Livestock plays a pivotal role in India's economy and society, contributing significantly to food security, rural livelihoods, agricultural productivity, and economic growth. As the world's largest milk producer, India contributes 25% of the global milk output. However, the livestock sector also accounts for nearly one-third of global anthropogenic methane emissions, making it a key contributor to climate change. In India, 42% of methane emissions are from buffalo, 41% from indigenous cattle, and 17% from crossbred cattle. Among various causes, repeat breeding in livestock significantly adds to methane emissions but has received limited research attention. While feed quality, animal diets, and manure management have been well studied, reproductive inefficiencies remain underexplored in emission control strategies. This study explores how digital innovations can help identify reproductive challenges, particularly repeat breeding and reduce methane emissions. A study across 30 villages in Coimbatore and Erode districts of South India assessed over 2,250 dairy animals from 470 small farmers using digital tools like the NITARA Farmer and Friend app. Key challenges, such as poor reproductive performance, low productivity, and poor health status were identified through detailed herd structure analysis. More than 20 WhatsApp groups were formed to share village-specific educational content based on these insights. The project uses NITARA, a digital dairy app, to analyse herd structure and share tailored solutions via WhatsApp. The effectiveness of WhatsApp as an information dissemination tool can be further scaled through farmer advisory centres, dairy cooperatives, and private sector partnerships to address productivity challenges in dairy animals. Additionally, 10 masterclasses and 35 animal health camps were conducted to address particular issues identified through data. These interventions led to a 55% reduction in repeat breeding cases and increased pregnancy rates from 26% to 48% within eight months. Optimised inter-calving intervals and improved reproductive performance have a direct impact on reducing methane emissions per unit of milk produced. The study highlights livestock's vital role in India's economy and global milk production, while also addressing its environmental impact, especially methane emissions. While feed and manure management have improved, reproductive inefficiencies like repeat breeding remain neglected for their impact on climate change. By using digital tools like the NITARA app and WhatsApp groups, the study effectively bridged this gap enabling real-time data collection, advisory support, and educational outreach. Targeted interventions and health camps significantly improved reproductive outcomes, demonstrating the power of integrating technology with field-level action to drive sustainable livestock management. By analysing herd structure, the project was able to identify unproductive animals, such as repeat breeders and chronically ill livestock. By enabling targeted interventions, the project improved herd efficiency and reduced methane emissions per unit of production. The findings underscore the importance of combining technology with grassroots action to address livestock reproductive challenges. In conclusion, this study highlights the potential of integrating digital innovations with evidence based field interventions to improve livestock health and productivity. It serves as a scalable model for sustainable livestock management, demonstrating that improving reproductive performance can contribute meaningfully to climate change mitigation while supporting farmer livelihoods.

418 Empowering dairy entrepreneurship to boost productivity, enhance livelihoods, and reduce methane emissions in Tamil Nadu

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Animal husbandry is a cornerstone of livelihoods for rural communities in Tamil Nadu, particularly for small-scale and landless households. Livestock farming contributes nearly 30% to rural household incomes and provides significant employment opportunities, especially for women. Despite being one of India's top eleven milk-producing states, Tamil Nadu lags behind Andhra Pradesh and Karnataka in total milk production, with an average dairy productivity of 5.63 liters per animal per day—well below the national average of 7.61 liters. Smallholder dairy farmers face persistent challenges, including high feed costs, green fodder shortages, low productivity, and limited access to veterinary and extension services. This project aims to create a robust dairy entrepreneurship ecosystem in Tamil Nadu to address these challenges and improve productivity, profitability, and sustainability. It will train and equip rural youth, dairy entrepreneurs, and startups with technical and business expertise, enhancing the delivery of high-quality inputs and climate-resilient advisory services to dairy farmers. Focused on Erode and Coimbatore districts, the initiative has established two dairy business incubation centres to foster innovation and entrepreneurship. From an initial pool of 1,500 candidates identified through 10 Taluk-level workshops, 400 were shortlisted, leading to the final selection of 112 entrepreneurs across five domains: value addition, nutrition, breeding, housing and health, and machinery & equipment. The cohort includes 26 established entrepreneurs, 78 new entrepreneurs, and 8 startup innovations. This diverse mix ensures a balanced representation of experience and creativity, driving scalable and resilient solutions within the dairy sector. The incubation centres serve as hubs offering mentorship, infrastructure, and linkages to industry and markets. Entrepreneurs gain access to solutions such as high-quality fodder seeds, silage, ration-balancing services, milking machines, and veterinary products, including vaccines and sexed semen. Digital tools for monitoring animal health and productivity further strengthen the value chain and reduce inefficiencies. The project aims to support 10,000 farmers by addressing critical gaps in feeding, breeding, and animal health. Entrepreneurs will play a central role in delivering these services, helping farmers adopt practices that improve milk yields and animal well-being. These interventions will significantly reduce greenhouse gas emissions per unit of milk produced. Enhanced feeding practices and animal health management reduce enteric methane emissions by improving digestive efficiency and lowering disease incidence – two major contributors to emissions in the dairy sector. In doing so, the initiative contributes to improved rural livelihoods, nutritional security, and environmental sustainability. By aligning productivity gains with climate mitigation strategies, the project supports Tamil Nadu's ambition to lead in climate-smart dairy development. With its strong governance framework and vibrant startup ecosystem, the state offers a conducive environment for scaling such innovations. This initiative provides a replicable model for sustainable dairy entrepreneurship that can be extended across India. It strengthens rural economies, promotes gender-inclusive growth, and positions the dairy sector as a key driver of sustainable and climate-resilient economic development.

467 Practical experience mitigating methane with sustainability indexes

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National breeding programs in Australia, Ireland, Canada, and New Zealand have integrated sustainability-focused genetic selection tools to enhance the environmental efficiency and economic viability of livestock production. Sustainability indexes and sub-indexes, such as the carbon sub-index in Ireland's Economic Breeding Index, Australia's Sustainability Index, New Zealand's Cool Sheep Programme, and Canada's modernized Lifetime Performance Index, which includes a direct methane trait within its efficiency sub-index, incorporate traits that optimize feed efficiency, reduce methane emissions, and improve animal health and longevity. These tools provide an opportunity for genetic mitigation of livestock emissions and have been adopted to varying degrees across different agricultural systems. This study reviews the application of sustainability indexes in practice, evaluating the development, adoption, and impact of these breeding programs on emissions reduction. Real-world examples of industry-available tools are explored to assess their effectiveness in reducing greenhouse gas (GHG) emissions and identify barriers to widespread implementation. One of the primary obstacles to adoption is the absence of formal policy mechanisms that recognize and quantify emissions reductions achieved through genetic selection. Addressing this issue requires collaboration among policymakers, industry stakeholders, and researchers to ensure that genetic progress is acknowledged in national greenhouse gas inventories and sustainability policies.

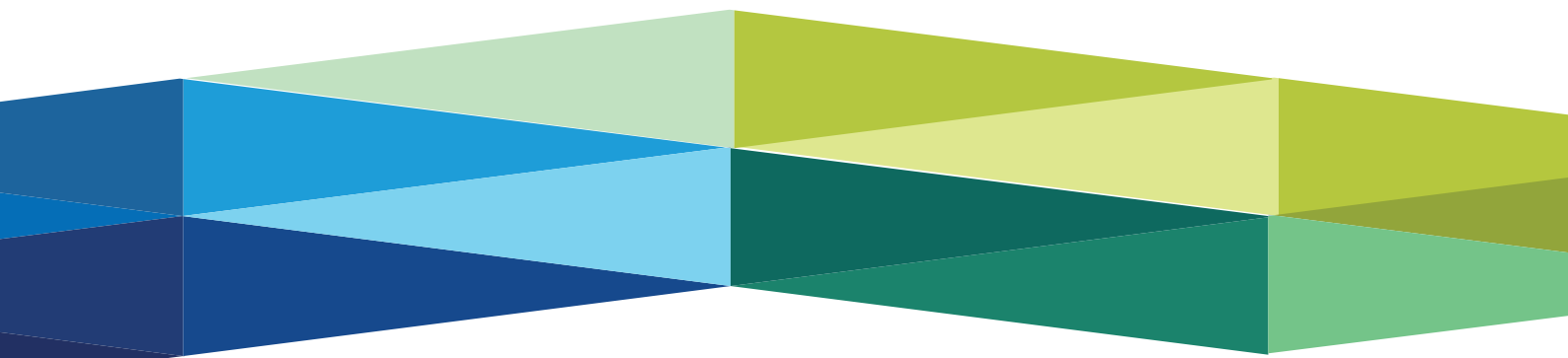
Motivation for the adoption of sustainability indexes is increasing in high-income countries. However, this motivation could be further enhanced if there were clear, auditable, and scientifically robust pathways for recognizing emissions reductions due to genetic selection. Establishing transparent methodologies for quantifying genetic contributions to emissions reduction would support national policy setting and facilitate supply chain agreements with processors that reward sustainable breeding decisions. Several genetic selection strategies have been developed to target reductions in GHG emissions while maintaining economic viability. This can be accomplished by targeting genetic progress in a specific trait, such as methane emissions, or by optimizing selection indexes to balance economic gain with environmental impact. Various national breeding programs have taken different approaches to incorporating emissions-related traits into their selection criteria. While direct methane breeding values remain challenging to implement in many cases, alternative strategies have been developed to optimize current index traits for emissions reduction. Recent methodologies have quantified the independent impact of existing traits on system-wide emissions. These approaches have expanded beyond methane to incorporate broader sustainability factors, including acidification and eutrophication potential. Although breeding programs differ in their specific objectives and constraints, research suggests that 40–50% of the maximum achievable emissions reductions can be realized with only modest sacrifices in economic gain due to the elliptical response properties of selection indexes. The most effective long-term strategy involves the integration of a direct methane trait with enhanced selection for other traits contributing to environmental sustainability. In the short term, emissions coefficients can be incorporated into existing indexes, providing farmers with an immediate tool for reducing emissions while maintaining profitability. Current breeding strategies demonstrate a strong commitment to sustainability, but greater adoption requires clear recognition of genetic progress within emissions inventories and sustainability policies.



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Theme 3: Rumen microbial genomics and nutrition



24 Rumen microbiome manipulation at early life reduced methane emission and improved digestibility in goats

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Rumen microbial ecosystem is rather resilient to change once the rumen is matured. Nutritional interventions and/or inoculants applied early in life modify the bacterial population colonizing the rumen and can help in reducing the methane emission and improving feed efficiency later in life. Effect of supplementation of essential oils (EEO) in the dam and early life of kids on rumen microbiome, methane emission and nutrient digestibility at a later stage of life was studied in Barbari goats. Sixteen adult (4m±7d pregnant) female Barbari Goats (Avg B.Wt.31.95±2.09 Kg) carrying twins were divided into two groups (CON and SUPP) of eight each as per completely randomized design. Kids from non-supplemented (Control) group of dam were divided into two groups (Con- and Con+) while kids from supplemented group of dam were divided into two groups (Trt- and Trt+). Supplementation of essential oils was done during the last month of pregnancy in SUPP group of dam and kids of Con + and Trt+ group up to weaning while no supplementation was done in CON group of dam Con – and Trt- group of kids. Animals were individually penned. Dam and kids were fed with concentrate pellet, gram straw and Moringa leaves to meet their nutrient requirement. At 5-6 months of age rumen liquor was collected from the kids using stomach tube, DNA was extracted to study rumen microbiota. A metabolism trial was conducted with all four groups of kids and nutrient digestibility and nitrogen balance was studied. *In vivo* methane emission from the kids group was measured using standardized Sulphur hexafluoride (SF₆) technique. Whole metagenome analysis was carried out using Illumina sequencing of rumen fluid samples as per CSIRO protocol. Study revealed *Bacteroidota* as top phyla in the entire kid groups followed by *Pseudomonadota*. Top genera were *Prevotella*, followed by *Escherichia* in all the four groups of kids. The % distribution of *Methanobrevibacter*, responsible for methane production was different in all the four group of kids. The distribution was lowest in Trt + group reflecting the effect of supplementation. Dry matter, organic matter and acid detergent fibre digestibility was significantly (P<0.05) higher in treatment group of kids while crude protein digestibility and nitrogen balance was statistically similar among all the kid groups. Mean methane production (g/day) was 15.68% less per day, 11.18% less per kg dry matter intake and 16.64% less per kg digestible dry matter intake basis in Gr T+. The present study concluded that supplementation of essential oils during early phase of life can modulate the rumen microbiome towards reduced methane emission and increased nutrient digestibility.

51 Microbiome signatures and pathways in Japanese Black Cattle by fertility levels

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The rumen microbiome is crucial for cattle health, facilitating the breakdown of complex carbohydrates into volatile fatty acids and essential nutrients. This diverse microbial community significantly influences cattle growth and productivity by optimizing feed conversion and metabolic processes. Our study examined the effects of geographical location and fertility on rumen microbial communities in Japanese Black cattle, employing 16S rRNA gene amplicon sequencing to analyze samples from 32 cattle at Togo and Oguri farms in Aichi Prefecture, Japan. The cattle were grouped into low- and normal-fertility categories based on artificial insemination outcomes, allowing us to explore how microbiome variations are influenced by both site and fertility. Rumen fluid samples were collected, processed, and the DNA extracted for sequencing the V3-V4 regions of the 16S rRNA gene. We utilized the Divisive Amplicon Denoising Algorithm 2 (**DADA2**) and Quantitative Insights into Microbial Ecology 2 (**QIIME2**) pipelines for bioinformatics analysis. The amplicon sequence variants (**ASVs**) were classified using the classify consensus-vsearch method against the latest SILVA database (version 138-99). Functional pathways were then analyzed using PICRUSt2, and further data analyses were conducted using R software with packages such as "phyloseq," "vegan," "MicrobiotaProcess," "coin," and "mixOmics." The sparse Partial Least Squares Discriminant Analysis (**sPLS-DA**) identified microbial taxa and pathways linked to fertility status. Our findings show that geographical location significantly impacts microbiome composition ($P=0.03$), with distinct microbial profiles between farms. Although fertility had a less pronounced effect, it still noticeably influenced microbial diversity and composition. Core microbiota, such as *Bacteroidota* and *Firmicutes*, were prevalent in all samples. Notably, taxa like *Fibrobacterota* and *Negativicutes* varied between sites. Metabolic pathway analysis revealed significant differences between fertility groups, with the sPLS-DA model highlighting pathways such as Nicotinate Degradation I and Sucrose Degradation IV as critical discriminators of fertility outcomes. Our findings demonstrate that site and fertility-related variations in the rumen microbiome significantly influence microbial diversity and functionality, which in turn impacts metabolic processes essential for cattle health and productivity. Notably, the rumen microbiome plays a critical role in the fermentation of feed, which is a major source of methane - a potent greenhouse gas. Variations in microbial communities, as observed in our study, could lead to differences in methane emissions among cattle. Therefore, understanding these microbial profiles provides a pathway to potentially mitigate GHG emissions through targeted microbiome manipulations. By optimizing the microbiome for reduced methane production, this research not only contributes to animal health and fertility but also offers a sustainable approach to lowering the environmental footprint of cattle farming.

86 Coenzyme M and methyl-coenzyme M reversed methanogenesis inhibition by 2-bromoethanesulphonate but not 3-nitrooxypropanol or bromoform

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Chemical inhibitors of methanogenesis are the most potent strategy to mitigate enteric methane emissions but there is variation in their efficacy in vivo and in the sensitivity of methanogens to inhibitors. We propose that variation in the efficacy of inhibitors of methanogenesis is related to their mechanism of action. 2-Bromoethanesulphonate and 3-nitrooxypropanol inhibit methyl-coenzyme M reductase by being structural analogues of coenzyme M and methyl-coenzyme M, respectively, whilst bromoform blocks the transfer of a methyl group attached to a cobamide to coenzyme M. We hypothesised that excess coenzyme M and methyl-coenzyme M would reverse methanogenesis inhibition by 2-bromoethanesulphonate and 3-nitrooxypropanol, respectively, and not by bromoform. Secondly, some methanogens lack the complete set of genes necessary to synthesise coenzyme M and thus require taking up external coenzyme M. We thus further hypothesised that supplementing coenzyme M to mixed rumen cultures would stimulate methanogens that need to take up coenzyme M, thereby favouring the transmembrane transport of its analogue 2-bromoethanesulphonate and enhancing its efficacy. Two rumen in vitro experiments were repeated in three different weeks with inoculum obtained from two non-pregnant, non-lactating Holstein cows incubated separately. In Experiment 1, mixed rumen cultures were supplemented coenzyme M at 0 or 1 µM in four 24-h serial incubations, with each culture donating inoculum to the next. In the fifth serial transfer, each culture received 25 µL distilled water, or 25 µL 2-bromoethanesulphonate, 3-nitrooxypropanol, or bromoform at 5 µM final concentration. In turn, each inhibitor treatment received distilled water or coenzyme M at 1 mM. Responses after five serial transfers were analysed as a function of supplementing coenzyme M in serial transfers 1 to 4, methanogenesis inhibitors and coenzyme M spiking in serial transfer 5, double interactions, and the random effects of inoculum and week. In Experiment 2, 48-h rumen batch cultures received the same inhibitors as in Experiment 1, each with distilled water or methyl-coenzyme M at 1 mM. Responses were analysed as a function of methanogenesis inhibitors, methyl-coenzyme M spiking, their interaction, and the random effects of inoculum and week. Overall, coenzyme M and methyl-coenzyme M reversed the inhibition of methanogenesis by 2-bromoethanesulphonate but not by 3-nitrooxypropanol or bromoform. In Experiment 1, supplementing coenzyme M in the first four serial transfers did not affect methane production. 3-Nitrooxypropanol and bromoform increased accumulation of dihydrogen and ethanol, whilst 2-bromoethanesulphonate only increased dihydrogen accumulation in Experiment 2 in the absence of methyl-coenzyme M. Bromoform, and 3-nitrooxypropanol in Experiment 1, increased methanol accumulation. Bromoform consistently decreased acetate concentration, whilst the effects of inhibitors on propionate and butyrate were variable. Coenzyme M and methyl-coenzyme M may compete with 2-bromoethanesulphonate for the active sites of methyl-coenzyme M reductase and coenzyme M transmembrane transporter, although the significance of this competition at in vivo extra and intracellular concentrations of coenzyme M is unknown. Consistent with previous research, our findings suggest that 3-nitrooxypropanol diffuses across membranes and inhibits methyl-coenzyme M reductase bound to coenzyme M or methyl-coenzyme M.

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108 Differences in the rumen microbial profile of dairy and suckler bred beef steers

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Methane originating from ruminant livestock, primarily produced as a by-product of the rumen microbial fermentation of feed, accounts for ~30% of global anthropogenic methane emissions. The effect of dietary management on both the rumen microbial profile and subsequent methanogenic output of livestock has been well established. More recently, differences in the composition of the rumen microbial profile of high and low methane emitting ruminants have previously been observed. However, to date there has been little comparison of the composition of the rumen microbial profile of divergent genotypes of cattle. Therefore, the aim of this study was to characterise the composition of the rumen microbial profile of Dairy or Suckler bred beef steers, having previously undergone detailed measurements of feed intake, feed efficiency (**FE**), carcass output and enteric emissions during an intensive finishing period. Rumen fluid samples were successfully obtained from 76 steers (Dairy n = 43; Suckler (Late-maturing sired) n = 33) offered the same total mixed ration (**TMR**; 77% concentrate and 23% forage) *ad libitum* during an indoor finishing period (82-102 days length), using a trans-oesophageal sampling device (FLORA rumen scoop). Rumen microbial DNA was extracted with 16S rRNA amplicon libraries generated and sequenced on the Illumina MiSeq. Sequence data was analysed using the DADA2 pipeline and microbial taxonomies classified using the SILVA (v.138) data base. Differences in the overall composition of the rumen bacterial and archaeal community were assessed using a PERMANOVA based on Bray–Curtis dissimilarities and 9,999 permutations, with cattle genotype included as fixed effect. Animal performance, enteric emissions data and microbial diversity (bacteria and archaea; Shannon Index) were assessed in SAS using the Proc Mixed function, with genotype and contemporary group (n=3) included as fixed and random effects, respectively. No difference in initial or final body weights were observed between genotypes ($P>0.05$). Dairy steers had a higher dry matter intake, daily production of both methane and carbon dioxide in comparison to Suckler steers ($P<0.05$). Both the overall composition of the rumen bacterial and archaeal community composition were impacted by cattle genotype ($P<0.01$). No difference in the diversity of the bacterial community structure, at the ASV level, were observed. However, Suckler steers had a less diverse archaeal community in comparison to the Dairy genotype ($H = 1.81$ vs. 1.98 ; $P < 0.01$). Based on the analysis conducted as part of this study, the composition of the rumen microbial profile has been shown to differ between two divergent genotypes of cattle. Further research will be required to determine the contribution of host genetics and early life management practices to the apparent differences in the rumen microbial composition of Dairy and Suckler beef genotypes of cattle.

248 Ruminal microbiome diversity in beef cattle grazing on traditional pastoral system from Colombian Caribbean region

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Latin America is emerging as a world provider of animal protein production, driven by the growing global demand for food. Colombia currently has around 16.6 million heads of beef cattle, raised primarily in grazing-based production system across meadows ecologically diverse, that could have effect of modulation of the ruminal microbiome structure, which plays a well-recognized key role in feed efficiency, nutrients metabolism and energy extraction. The aim of the present study was seeking the ruminal microbiome diversity of beef cattle across subregions of Cordoba department, which is characterized by temperatures ranging from 21 to 35.5 °C, relative humidity between 77-83% and monthly precipitation between 25 to 225 mm. Cattle grazed on a variety of forage species, mainly represented by *Dichanthium aristatum*, *Bothriochloa pertusa*, *Urochloa humidicola*, *Megathyrsus maximus sp* and *Cynodon nlemfuensis*. This study analysed ruminal fluid samples collected from 66 beef-cows grazing in traditional pastoral system distributed across six subregions: Alto Sinú (**AS**, 86 m.a.s.l), Sinu Medio (**SM**, 30 m.a.s.l), Bajo Sinú (**BS**, 50 m.a.s.l), Costanera (**C**, 43 m.a.s.l), Sabana (S, 78 m.a.s.l), and San Jorge (S, 77 m.a.s.l). The rumen metagenome sequences were obtained using 16S rRNA gene amplicon sequencing. The sample reads were processed using QUIIME2 and DADA2 software to obtain the precleaned reads and amplicon sequence variant list (**ASV**), respectively. ASV were mapped using Kraken2. Data analysis was conducted using R software. Alpha diversity metrics, Shannon diversity index and ASVs were calculated using Phyloseq package. Bray-Curtis was applied to determine Beta diversity. Multivariate analysis using principal component analysis (**PCA**) and Partial Least Squares Discriminant Analysis (**PLS-DA**) were performed to know if exists an associate cluster to subregions evaluated. Experimental procedures for this study were approved by the AGROSAVIA Honorary committee on Ethics in the Use of Animals. Results showed that Alpha and Beta diversity were not significantly different between subregions. However, the C, AS, and MS subregions presented the highest variability in the distribution of observed species (2,000 to 5,000). At the same time, the Shannon index demonstrated high species diversity (greater than 3), but no clear differentiation between regions was observed. The PLS-DA showed considerable overlapping between regions, only the 14% of variance was explained by the model (X variate1: 8% and X variate 4: 6%). This pattern was reinforced by the PCA, which clearly showed the overlapping between regions (PC1:15% and PC2: 9%). Thus, the apparent separation of AS and MS was related to individual sample variation and specific microbiological composition. Loadings for the PLS-DA model allowed identify highlights key microbial species across subregions. Negative contributions were observed for *Gastranaerophilales*, *Pedospaeraceae*, and *Oligosphaeraceae* taxa, associated to AS and *Endomicrobiaceae* associated to SJ. In the case of C and SM positive contributions were shown by *Desulfovibrionaceae* and *Desulfobulbaceae*, respectively. In conclusion, the total variance explained across subregions was less than 24%, suggesting that further multidimensional analyses are required to understand comprehensively the microbiome complexity and its potential for improving cattle performance in way to enhance the reliability of the results and design of region-specific feeding practices.

297 Effect of breed and grass silage quality on rumen microbiota in sheep

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Enteric methane (**CH₄**) is the most important emission source from sheep production, accounting for around 4% of global greenhouse gas emissions from livestock. Enteric methane is a result of methanogenesis, where hydrogen reduces CO₂ by methanogens. The rumen microbiota can be influenced by several factors, such as diet (e.g., forage quality) and breed, and hence enteric CH₄ production. This study compares rumen microbiota for two sheep breeds offered two silage qualities. The two breeds were the Old Norwegian Spæl (**ONS**), a light rural breed and the Norwegian White Sheep (**NWS**), which is a modern composite breed selected for high production. The experiment followed a 2x2 factorial design, where adult dry ewes (n=40, 20 of each breed) of the two breeds were given two contrasting grass silage qualities (Early vs. Average according to cutting time; difference 15 days; 7.12 vs 5.74 MJ net energy lactation per kg dry matter). The ewes were kept in individual pens and fed *ad libitum*. The experiment lasted for six weeks in two periods (one week of adaptation and two weeks with CH₄PAC measurements for each quality). The ewes were randomly grouped and each group assigned to one of the two qualities for the first period and switched to the other for the second.

At the end of each period, rumen fluid samples were collected from 24 ewes (12 of each breed) to assess microbiota composition through 16S rRNA gene sequencing. The ewes were kept off feed for a minimum of one hour before samples were taken using an orogastric tube. The fluid samples (minimum 30 ml, maximum 60 ml) were immediately frozen using liquid nitrogen before storage at -80 °C. Results showed that the ewes fed Early cut silage had lower enteric CH₄ emissions compared to those fed Average, and that the ONS had lower CH₄ emissions compared to NWS (both corrected for DMI). The analyses of rumen fluid revealed differences in community structure between silage qualities for both breeds. Further, the Principal Component Analysis (**PCA**) showed that the bacterial communities were distinct for the two silage qualities, while no clear difference was found between the breeds. This distinction was also evident in terms of diversity and richness, as well as number of identified operational taxonomic units (**OTUs**), which was higher for the early cut silage compared to the late cut silage. Differences in rumen microbiota may contribute to explaining contrasts in CH₄ production between the two silage qualities. However, the short-term study only analysed the microbiota composition which may not allow for a long-term assessment of microbiota dynamics and their impact on CH₄ production. Additionally, the study did not explore the functional aspects of how these microorganisms contribute to CH₄, which should be further investigated. The observed breed differences in CH₄ production may also be related to rumen passage rate, NDF digestibility, fermentation rate, rumen size etc., and merit further research.

316 A metagenomic catalogue of the ruminant gut archaeome

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While the ruminant gut archaeome regulates the gut microbiota and hydrogen balance, it is also a major producer of the greenhouse gas methane. However, ruminant gut archaeome diversity within the gastrointestinal tract (**GIT**) of ruminant animals worldwide remains largely underexplored. Therefore, the objective of this study is to construct a gut archaeal genome catalogue for ruminants and investigate their distribution and functional roles across different ruminant species and gut regions. Here, we construct a catalogue of 998 unique archaeal genomes recovered from the GITs of ruminants, utilizing 2270 metagenomic samples across 10 different ruminant species, which were primarily sourced from public databases such as NCBI and EMBL-EBI. Most of the archaeal genomes (669/998 = 67.03%) belong to *Methanobacteriaceae* and *Methanomethylophilaceae* (198/998 = 19.84%). We recover 47/279 previously undescribed archaeal genomes at the strain level with completeness of >80% and contamination of <5%. We also investigate the archaeal gut biogeography across various ruminants and demonstrate that archaeal compositional similarities vary significantly by breed and gut location. The catalogue contains 42,691 protein clusters, and the clustering and methanogenic pathway analysis reveal strain- and host-specific dependencies among ruminant animals. We also find that archaea potentially carry antibiotic and metal resistance genes, mobile genetic elements, virulence factors, quorum sensors, and complex archaeal viromes. Overall, this study establishes the first systematic and comprehensive catalogue of archaeal genomes in ruminants, serving as an invaluable resource repository for archaeal research. It provides a foundational platform for enhancing our understanding of archaeal ecology and developing targeted strategies to regulate methane production in ruminants.

318 Assessing the impact of feeding *Plantago lanceolata* L. on the rumen microbiome of dairy cows

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Improving the productivity and sustainability of pasture based production systems is a key focus for pasture fed ruminant agriculture. Plantain (*Plantago lanceolata* L.) has demonstrated potential as a plant species capable of improving sustainability through altering pathways of nitrogen (N) excretion. However, the role of the rumen microbiome in impacting this N partitioning is currently unknown.

Eight lactating multiparous Holstein-Friesian dairy cows were selected in a complete 2 × 2 crossover design. Treatment groups were balanced for parity, pre-study milk yield, milk fat concentration, milk protein concentration, days in milk, body weight, and body condition score. Treatment groups were offered one of two cut forages, perennial ryegrass (PRG; *Lolium perenne*) and white clover (WC; *Trifolium repens*) (PRGWC) or PRG, WC, and PL (PLA). Cows had *ad-libitum* access to their cut forage and were offered 2.75 kg DM concentrate/cow per d. Each period consisted of 16 d of dietary acclimatization followed by 5 d of measurements (water intake, dry matter intake (DMI), milk production and composition, urine, and feces; 21 d period). At the end of each 5 d measurement period, a rumen sample was collected using an oesophageal sampler. Following this DNA was extracted and 16S rRNA region was sequenced using Nanopore sequencing.

Total DMI was greater for cows offered PLA (22.9 vs. 21.4 kg DMI/cow), and cows tended to produce a greater yield of milk and milk protein when offered PLA. Urinary N concentration and excretion were lower from cows offered PLA (170 vs 157 g/d and 0.36 vs. 0.45 % respectively). Cows fed PLA tended to have reduced diversity based on Observed and ACE measures of diversity. β -diversity calculated using a Bray-Curtis distance matrix and a PERMANOVA determined that microbial composition differed between cows fed PLA and PRGWC. While no taxonomic differences were identified at either Phylum or family level a number of differentially abundant genera were identified. Within the family *Lachnospiraceae*, *Lachnoclostridium*, *Coprococcus*, *Anaerobutyricum* were significantly increased in cows fed PLA while *Roseburia* was reduced. In addition, within the family *Oscillospiraceae*, *Faecalibacterium* was increased while *Fastidiosipila* was decreased following the feeding of PL. Ongoing analysis is focused on establishing associations between identified taxonomic differences and production traits.

Differences in both α and β -diversity were identified between cows fed either PLA compared to fed PRGWC with lower diversity found in cows fed PLA. In addition, changes of taxa within the *Lachnospiraceae* family in particular contrasting abundance of *Roseburia* and *Lachnoclostridium* may be responsible for phenotypic differences following the feeding of the different forages. Further work is ongoing to better understand these changes.

347 Methane emissions, productivity parameters, and rumen microbiome across development stages of East African Boran cattle

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Cattle production in East Africa is mainly pasture-based, supporting livelihoods and food security. Accurate measurement of enteric methane (**CH₄**) is crucial for improving national greenhouse gas (**GHG**) inventories and developing effective mitigation strategies. Since CH₄ emissions are closely linked to feed intake, this should also be measured accurately. While understanding the host-microbiome system may enhance feed efficiency and reduce emissions, the reasons for variations in rumen microbiome composition among individuals on the same diet remain unclear. The objective of this study is to investigate changes in rumen microbiome structure and functions, enteric CH₄ emissions, and production efficiency and their associations through animal development in Boran East African beef cattle in an extensive grazing system at the Kapiti Research Station in Kenya. The animal developmental stages studied here are based on age, with animals grouped into three age-based cattle categories: 1. pre-weaning (6 weeks to 6 months), 2. early post-weaning (7 to 12 months), and 3. adults (13 to 24 months). The entire study will last for two months starting in June 2025. Sampling will be staggered with measurements being conducted on each animal group at the time (7 animals/cohort) with a total of 14 animals in each age group. Composite samples of the offered pasture will be collected for dry matter and chemical composition analyses. Dry matter intake (**DMI**) will be assessed using titanium dioxide (**TiO₂**) as an external marker, with faecal sample collection by grab sampling from the rectum. Enteric CH₄ emissions from experimental animals will be measured using the sulphur hexafluoride (**SF₆**) gas-tracer technique and will be used to determine specific emission factors (**EF**) for each cattle category. Rumen fluid from each cattle will be collected using a stomach tube connected to a mechanical pumping unit and stored in sterilized containers. Aliquots of the rumen samples will be used to analyze rumen fermentation products, including volatile fatty acids and ammonia. Shotgun metagenomic sequencing will be performed on the rumen fluid samples, and the sequencing data will be assembled using metagenomic binning techniques to profile the gut microbial composition and function. Host genetic analyses will be conducted using hair samples collected. Live animal weights will be measured along the trial. The results of this prospective study, unique in its kind in East Africa, will be presented to support climate-smart livestock management practices. Expected research outcomes include: 1. Successful implementation of the SF₆ gas-tracer technique for measuring enteric CH₄ emissions in local conditions, 2. Development of age-specific enteric CH₄ emission factors for East African Boran cattle, 3. Profiling of rumen microbiome of local cattle breeds across animal developmental stages, and 4. Training generative models of microbial abundances, phenotypic variables, and animal metadata to unravel host-microbiome interactions, linking the rumen microbiome to CH₄ emissions and performance parameters across animal developmental stages.

370 The Greener Cattle Initiative: a research program to inform and enable enteric methane mitigation strategies

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Globally, methane emissions account for 40-45% of greenhouse gas emissions from ruminant livestock with more than 90% of these emissions arising from enteric fermentation and 10% from manure. Ruminants have evolved a synergistic relationship with a dense and complex microbial community in the rumen to efficiently degrade and ferment dietary polymers and produce CH₄ as a natural and essential consequence of digestion. Numerous CH₄ mitigation strategies for ruminants are emerging but despite the dogma that production of CH₄ is a waste of energy that could be redirected back to the animal if abated, researchers are not observing predictable co-benefits from these approaches. Central to anaerobic fermentation in the rumen are the energetic and kinetic processes involved in H₂ production and subsequent H₂ utilization mainly via reduction of CO₂ with H₂ to form CH₄. Thus, knowledge of the rumen microbiome is key to understanding these mechanisms. For this purpose, an international consortium of rumen biologists was developed and obtained funding from the Greener Cattle Initiative through the Foundation for Food and Agricultural Research to carry out this critical research program. The consortium comprises six international Research Hubs that are collectively working to identify and quantify routes and fluxes of electron flow and thus their potential for diverting energy away from CH₄ towards more nutritionally and sustainable benefits. In addition, the consortium is working to identify and fully understand microbial system changes related to CH₄ formation and impacts of CH₄ mitigation at a mechanistic 'omics' level.

In this innovative research program, we are focused on understanding microbiome composition, adaptation and function and the impact that methane mitigation has on applied ruminant production. In depth mechanistic studies in which we use dual flow continuous culture systems to establish fermentation conditions that have graded levels of CH₄ production and performance using CH₄ inhibitors such as BES and 3-NOP, followed by studies either at the herd level or in metabolism chamber-calorimeter type of studies with fewer animals. Importantly, we will couple measurements of the microbiome, including ruminal ciliate protozoa, as well as key metabolites with dissolved gasses (dH₂) that contribute to the high and low CH₄ emitting phenotype in order to identify promising mitigation strategies that alter H₂ flow favorably resulting in reduced CH₄ emissions without loss of productivity in beef and dairy cattle. At one research hub, the use of synthetic microbiome communities have been created that recapitulate the rumen microbiome that results in high and low CH₄ emission states. At another research hub, studies based on the addition of alternative hydrogen utilizing bacterial species is being evaluated.

Multiple strategies all based on a thorough mechanistic understanding of these processes are essential since it is only with a comprehensive understanding of the biochemical and genetic mechanisms involved that a complex system such as the rumen can be successfully and predictably manipulated to achieve and exceed the global warming target. Our innovative systems approach will reveal these hidden mechanistic details missing from recent microbiome studies examining CH₄ reduction.

401 Exploring ruminal bacteria for methane mitigation: screening potential through VFA profiles

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Methane (**CH₄**), a byproduct of rumen fermentation, significantly contributes to greenhouse gas emissions and causes energy loss in livestock production. Strategies to reduce CH₄ emissions include optimizing rumen microbial processes by modulating fermentation pathways toward reduced CH₄ production, while simultaneously improving animal energy efficiency. Recent culturomics studies have expanded ruminal culture biobanks, offering valuable resources for exploring strain-level genomic variation, which remains largely underexplored. These strains are candidates for early intervention studies aimed at mitigating enteric CH₄ mitigation during the rumen developmental stage of acquiring the rumen microbiome. By exploring the full genetic diversity within these strains, particularly regarding their potential for volatile fatty acids (**VFA**) production, a better understanding can be gained of how specific genetic factors influence ruminal fermentation dynamics. VFAs, such as propionic and acetic acid play crucial roles not only in energy metabolism and rumen functionality but also in hydrogen (**H₂**) dynamics. Enhanced propionic acid production is linked to the redirection of H₂ away from methanogenesis, thereby reducing CH₄ emissions. Conversely, the production of acetic acid increases H₂ availability, which in turn supports the activity of methanogenic archaea. This study, conducted as part of the Greener Cattle Initiative (**GCI**) project, investigated the VFA profiles of rumen bacterial isolates, including various strains, to better understand their potential in influencing ruminal fermentation.

Ruminal bacterial isolates from a recently established biobank, obtained from bovine and ovine ruminal fluid through dilution-to-extinction and direct plating methods, were selected for VFA phenotyping based on insights from literature, focusing on their association with propionic acid ruminal production pathways. Selected pure cultures were grown in liquid M2 medium until they reached the stationary phase. Optical density and pH were individually measured and recorded for each isolate. After centrifugation, the supernatant was collected for VFA analysis via gas chromatography. The gas chromatograph (Agilent 8890) is equipped with a flame ionization detector and an Agilent HP-FFAP column (30 m, 0.53 mm). VFA concentrations are being quantified using a standard curve generated from known concentrations of pure VFA standards. An internal standard was added to the samples to ensure the accuracy and reliability of VFA quantification. The ruminal bacterial biobank currently contains 673 bacterial isolates, which were considered for the initial screening of potential candidates. Based on their partial 16S sequences, these isolates were classified into seven phyla: *Actinomycetota*, *Bacillota*, *Bacteroidota*, *Fusobacteriota*, *Pseudomonadota*, *Spirochaetota*, and *Thermodesulfobacteriota*. *Bacillota* and *Bacteroidota* were identified as the predominant phyla, both recognized for their key roles in rumen fermentation processes. A subset of isolates, including strains closely related to the genera *Megasphaera*, *Propionibacterium*, *Roseburia*, *Selenomonas* and *Sharpea* was initially selected for VFA characterization, and the analysis is currently underway. Ongoing phenotypic characterization of these isolates, including VFA profiling, will provide critical insights into the metabolic potential of novel rumen bacteria. By identifying strains with desirable VFA production profiles, this work contributes to the development of targeted strategies for mitigating CH₄ emissions and enhancing ruminal fermentation efficiency.

419 Co-development of the host immune system and the establishment of methanogens in ruminants in early-life

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Recent studies have identified that the main intervention to reduce enteric methane production is by direct impacting methanogenic Archaea in the rumen. Although feed additives are being developed, their application is still limited and not feasible in grazing systems at the moment. Vaccines represent a great potential since they don't require direct and frequent access to the animals so they offer a universal solution which is both scalable and cost effective, integrating seamlessly into existing farm management systems. However, with vaccination, we are faced with diverse problems, the main one being our scarce knowledge regarding the recognition and modulation of the symbiotic rumen microbiota by the animal host. The objective of this project is to describe in detail the colonization and establishing pattern of methanogenic archaea along the digestive tract of newborn calves and assess the impact on the immune system development. The potential maternal role in modulating the innate immune response will be also evaluated.

We are currently undertaking a trial to follow the microbiome colonization in neonatal calves (birth to weaning) and to assess its impact on the immune system response. Peripheral blood samples, digestive tract content, lymph nodes, and mucosal swabs are being collected to analyse the luminal and mucosal-associated microbiome. Blood, faecal, and colostrum samples from the mother are also collected to analyse the immune and microbiome profiles to eventually identify potential transmission to the newborns and the impact on immune response. The luminal and mucosal associated microbiome will be studied via shotgun metagenomic sequencing, placing special emphasis on methanogenic archaea. In parallel, colleagues at RVC will be studying the composition of peripheral blood and Peyer's Plaques will be assessed by flow cytometry and immunohistochemistry using bovine specific antibodies as well as qRTPCR for immune profiling/scRNASeq. Plasma samples will be prepared for cytokine, antibody proteomic, lipidomic and metabolomic analysis. The final step will be integration of all data into a network to assess important communication nodes between organs-immune-system-microbiome.

Preliminary results showed that there is early and substantial colonization of the gut from birth by methanogenic archaea. We will present the evolution of the different archaea groups establishing along the gut and the link with the immune innate response of the animal.

The study is conducted in collaboration between Spanish Research Council (**CSIC**, Spain) and Royale Veterinary College (**RVC**, UK) and is part of a larger program funded by the Bezos Earth Fund and Global Methane Hub that also support another project looking at identifying and characterizing specific antibodies needed to target methanogens effectively. The overall program aims to overcome significant scientific hurdles, providing a foundation for a global methane vaccine initiative.

422 Leveraging RNA interference technology to target methanogens for mitigation of enteric methane emissions in cattle

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Methane is a potent greenhouse gas that significantly contributes to global warming and climate change. Ruminants are key methane emitters, as it is a by-product of ruminal fermentation. In the rumen, methanogenesis is performed by archaea, among which *Methanobrevibacter ruminantium* and *Methanobrevibacter gottschalkii* are often identified as the predominant species. Previous attempts to lower methanogenesis have had limited success due to low efficacy and inconsistent effects. Understanding the metabolic pathways that influence the persistence and activity of ruminal methanogens is crucial for developing effective methane mitigation strategies for ruminants. Our previous study identified species-specific non-coding RNA (**ncRNAs**) from key rumen methanogens, presenting potential for exploring RNA interference (**RNAi**) technology for targeted methanogen manipulation.

The current study used *Mbb. ruminantium* M1 as the model organism to examine the feasibility of applying RNAi to manipulate its growth and potentially methane production. 1mL of *Mbb. ruminantium* M1 pure culture stock was inoculated into 5 mL of BY basal medium (a culture method for methanogens grown without direct H₂) in Hungate tubes which included 200 mM ethanol as the substrate. Mixed gas of NO₂/CO₂ at the ratio of 85:15 was sparged into the headspace of the tubes, and the cells were incubated at 37°C. Methane production from the culture was monitored using gas chromatography. Double stranded RNA (**dsRNA**) was synthesized using MEGAscript Kit (Invitrogen™, Carlsbad, CA), from the DNA template of the target gene which encodes for the RNA template used for RNAi interference. The quantity and quality of the generated dsRNA was measured using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and gel electrophoresis.

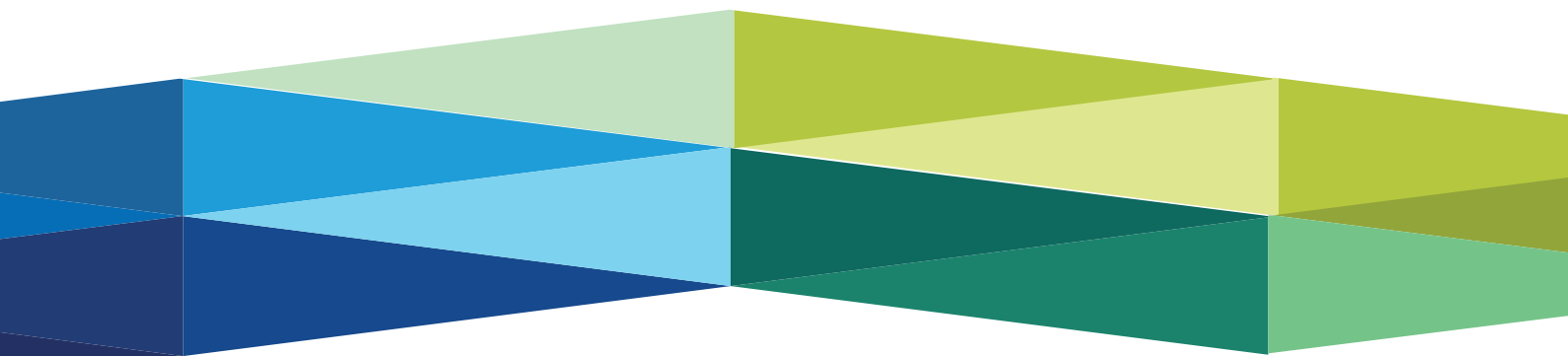
Using alternative ethanol-supplemented culture media and conditions, growth of *Mbb. ruminantium* M1 and methane production was observed and detectable. In two separate culture experiments, log phase was recorded at day 10, with 1.34 µmol/L methane gas produced in both experiments. With continuous monitoring, methane gas peaked at day 56, at concentrations of 1.44 µmol/L and 1.41µmol/L in each experiment, respectively. Gas production declined from day 68 in both experiments. To confirm successful dsRNA synthesis for RNA interference, the amplification and transcribed products were analyzed by agarose gel electrophoresis, and a sharp band of predicted molecular weight suggested the production of targeted dsRNAs. The dsRNAs are being evaluated for their ability to inhibit methane production from *Mbb. ruminantium* M1. The results demonstrated that culturing *Mbb. ruminantium* M1 without direct H₂ gas supply and costly infrastructure was feasible. The successful synthesis of dsRNAs using the DNA template of the target gene yields sufficient amounts for RNAi application. Once the efficacy of RNAi is assessed using *in vitro* fermentation assays, additional experiments will be conducted to further determine its ability to mitigate methane in cattle. These efforts contribute to exploring novel options to mitigate greenhouse gases on-farm and combat climate change.



9th GGAA
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5 - 9 October

International Greenhouse
Gas & Animal Agriculture
Conference

Theme 4: Manure management



15 Climate Change Adaptation Strategies in Manure Management in Kenya's ASAL Regions

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In Kenya's Arid and Semi-Arid Lands (ASALs), climate change poses significant challenges to agricultural productivity and food security, particularly in livestock systems where manure management is critical. This study investigates adaptation strategies for manure management that can enhance resilience in these regions while addressing environmental concerns. The research employs a mixed-methods approach, integrating field surveys, farmer interviews, and soil nutrient analysis to assess the effectiveness of various manure management techniques. Data collection is on-going, and findings will be available for presentation at the conference.

ASAL regions are characterized by unpredictable rainfall patterns, prolonged droughts, and increased temperatures, which adversely affect manure management practices. Traditional methods often lead to nutrient runoff, soil degradation, and heightened greenhouse gas emissions. To adapt effectively, farmers must embrace innovative strategies that optimize manure use, improve soil health, and safeguard water resources. This study employs a mixed-methods research design, integrating both qualitative and quantitative approaches. Field surveys are conducted among farmers in selected ASAL regions (Narok and Kajiado Counties in Kenya) to gather insights on current manure management practices and challenges. Structured interviews with agricultural extension officers and policymakers provide additional context on adaptation strategies and policy frameworks. Soil nutrient analysis is performed to assess the impact of different manure management techniques on soil fertility and moisture retention. Data analysis involves statistical techniques for quantitative data and thematic analysis for qualitative responses.

Preliminary findings indicate that manure management practices in ASAL regions vary widely, with traditional methods contributing to nutrient losses and environmental degradation. Farmers who have adopted composting and precision manure application techniques report improved soil moisture retention and crop yields. Agro-ecological practices, including agroforestry and cover cropping, show promise in enhancing soil health and mitigating climate-related risks. The adoption of anaerobic digestion systems remains limited due to initial investment costs, but households using biogas report reduced dependency on firewood and enhanced soil fertility from digestate application. Community-led knowledge-sharing initiatives emerge as a key factor in the successful implementation of sustainable manure management practices. The study highlights the critical role of innovative manure management strategies in enhancing climate resilience in Kenya's ASAL regions. Integrating precision manure management, composting, agro ecological practices, and biogas technology can significantly improve soil fertility, reduce environmental impacts, and support sustainable agricultural livelihoods. Policy interventions that provide financial support and training for farmers are essential for scaling up these adaptation strategies. Future research should explore long-term impacts and cost-benefit analyses of these approaches. The final results will be presented at the conference, contributing to discussions on sustainable agriculture and climate adaptation.

26 Do ammonia emissions from dairy farms impact nutrition and the environment? Evidence from meta analysis

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Globally and throughout Sub-Saharan Africa (SSA), livestock production produces greenhouse gases (GHG) and ammonia (NH₃) emissions, which lead to pollution of the air, water, and soil, and its impacts on the environment and nutrition of dairy cattle are concerning. When NH₃ concentrations in confined areas reach dangerously high levels, animals' health may suffer. Dairy manure contains nitrogen that may be converted and released as NH₃, which can cause respiratory issues in humans and animals and serve as an indirect source of N₂O. Understanding the effect of ammonia emissions from dairy farms on nutrition and the environment is critical to know the best strategies to adopt in reducing NH₃ and GHG emissions from livestock and producing livestock in a clean environment. It was against these backdrops that the study systematically collated and analyzed empirical published data on NH₃ emissions undertaken in critical dairy farms in Nigeria, SSA and globally. The study utilized meta-analysis and had to meet specific requirements before being added to the data set. The requirements were (i) Studies of dairy cows in vivo that reported emissions from dairy housing; (ii) Publications in English or French; and (iii) Mean reporting NH₃ emissions per cow per day. In addition, whereas the preliminary analysis used data from sixty (16) studies, the meta-analysis utilized verifiable data from eight (8) empirical studies reporting ninety (90) treatment means. The season and flooring type significantly affected NH₃ emissions. To determine the heterogeneity of mean NH₃ emission for nutritional effect analysis, the study employed the random effect method and the results were statistically significant ($P < 0.01$) at a 1% level of probability. Furthermore, random effect methods were extended to mixed-effect methods to take into consideration the heterogeneity in the available dietary and animal variables. The final mixed-effect methods, which separately considered dry matter intake, dietary crude protein, and milk yield, were able to explain 63% of the heterogeneity in NH₃ emissions. From the result, NH₃ emissions increased by 15.1 and 21.3 g cow⁻¹ d⁻¹ for every unit increase in dietary crude protein content (%) and dry matter intake (kg d⁻¹), respectively, while NH₃ emissions decreased by 6.2 g cow⁻¹ d⁻¹ for every unit increase in milk yield (kg d⁻¹). In conclusion, the study is critical in identifying sustainable ways of mitigating NH₃ and GHG emissions from dairy farms. Ultimately, the study have provided a deeper understanding of how best to reduce NH₃ and GHG emissions, increase milk production, promote animal welfare and produce dairy cattle in a clean environment.

39 Methane and nitrous oxide emissions from manure and response to farming system dynamics in Ethiopia

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Manure management in the dairy production contributes considerably to greenhouse gas (GHG) emissions in agriculture. Intensification of the sector led to increased volumes of manure accumulation, which emit GHGs like methane (CH₄) and nitrous oxide (N₂O). In order to contribute to global efforts to reduce CH₄ and N₂O emissions from manure management and utilization practices, reliable information is essential. The present study is therefore designed to generate country-specific improved (Tier II) emissions factors (EF) for manure CH₄ and N₂O based on animal energy requirements derived from animal performance and feed characterization data from about 2500 cattle in 480 households and three smallholder dairy farming systems in the Salale highland. Our result showed that there was significant variation in manure CH₄ and total N₂O EFs across the farming systems. The predicted EF value for adult dairy cows was 14.89, 11.68, and 3.19 kg CH₄ head⁻¹ year⁻¹, and 0.62, 0.53, and 0.21 kg N₂O head⁻¹ year⁻¹ for urban, peri-urban, and rural farming systems, respectively. The maximum manure CH₄ and N₂O EFs were reported for adult dairy cows in urban farming systems. Smallholder rural farming systems showed significantly lower CH₄EFs and N₂O for adult dairy cattle compared to urban and peri-urban farms. The manure CH₄EF estimates in this study are higher than IPCC's (2006) default values, while the N₂O EF estimates are lower than IPCC's (2006) default values for sub-Saharan Africa. Our findings can provide a baseline for the national emission inventory, which can be used to quantify future interventions that may reduce manure GHG emissions. This study suggests that increasing the proportion of manure used for composting and biogas instead of dung cake as fuel would result in an increase in organic manure and renewable energy sources on farms, thereby improving nutrient use efficiency and reducing greenhouse gas emissions.

41 Overview of household that produces poultry and uses manure in Gwagwalada Areas Council, Abuja Nigeria.

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Branckaert (quoted in Sonaiya, 1990) went on to define family poultry as "small flocks managed by individual farm families, in order to obtain food security, income, and gainful employment for women and children." And does family poultry producing meat and eggs, family poultry firms also create revenue via a number of additional and integrated farming activities. We are doing this research and examining some of the family poultry businesses in Gwagwalada, Abuja, Nigeria, in order to determine why the different faecal disposal and management strategies have become a concern and whether there are any currently available simple technologies for managing and turning this into a profitable business. We chose this by surveying middle- and lower-class family farms that raise layers and broilers for small-scale and commercial markets, with roughly thirty families. We also conducted oral interviews with individual households that raised poultry in the Gwagwalada areas councils, held focus groups, and made some physical observations, which were backed up by the pictorial representation. We went to these homes to get information on feedings, urine control, faeces removal, and management. Throughout the course of the various studies, fifty household field poultry keepers in various Gwagwalada areas will participate in oral interviews, where recoded questionnaires, observations, and supporting documentation will be recorded. This study reveals that few families engage in family poultry businesses, which are small-scale poultry keepers by households using family labour and, whenever possible, locally available feed resources, because the need to combat desertification and the issues of poverty levels seem concerning and uncalled for in the majority of developing countries. We found that while the homeowner provides the majority of the food for the hens, some are allowed to walk freely throughout the home compound and consume a large portion of their own food. The different cages of layers and broilers, some for personal use and some for commercial use, are housed in deep systems in a small cage that other hens build. In some places, stocking rates have risen to five birds, with one adult chicken bird in the lower cage and almost ten white chicks per square metre of floor space. Because they are not permitted to roam around much, some birds come into contact with excrement. Due to poor management and inappropriate waste removal, certain areas of the ecosystem have an unpleasant odour. Collections were difficult, and the odors systems aren't working well, which suggests that there aren't any good defecation habits that will support the facade's drainage systems. It breeds in many sorts of mosques and flies, which are hazardous for the house systems, and birds fly to consume the leftover food from the poultry pens. As for the watering systems and the need to minimize the various insects and the proper treatment of the poultry faeces, we also suggested good cleaning and management of the family poultry in Gwagwalada. Additionally, there are several poor enlisted water management systems. The vegetable producers buy the various excretions and incorporate them into their plantations.

69 Long-term effects of different cattle (*Bos taurus*) kraals occupancy durations on vegetation response

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Land degradation threatens ecosystems and biodiversity, particularly in semi-arid regions where continuous grazing worsens soil degradation. In these environments, sustainable grazing strategies are essential to reversing degradation. "Boma grazing," a traditional livestock management practice where animals are confined to small areas for short periods, has shown promise in restoring degraded rangelands. The concentrated livestock activity enhances soil fertility through trampling and manure deposition, which accelerates nutrient cycling, breaks up compacted soil, and facilitates seed germination. The ecological benefits of boma grazing, however, depend on the duration of livestock occupancy. Shorter stays may promote rapid vegetation recovery, while extended stays can lead to deeper soil modifications. Prior research suggests that even brief boma durations (4–7 days) improve vegetation by increasing grass cover and reducing bare ground. However, only longer boma durations (14–28 days) have been linked to significantly higher wildlife activity, likely due to the prolonged presence of nutrient-rich soils and preferred forage. This study investigates whether shorter-duration bomas (4–7 days) eventually develop into functional glades that attract wildlife over time. To assess this, we surveyed 25 abandoned experimental boma sites established in 2011, categorized into five treatments based on occupancy duration (0, 4, 7, 14, and 28 days). Each treatment was replicated across five different blocks to account for environmental variability. To evaluate vegetation recovery, we conducted detailed surveys using two 150 m and two 50 m transects per site. Along each transect, 1×1 m quadrats were placed every 10 m to measure bareness, vegetation cover, species diversity, and abundance. Wildlife activity was monitored using 25 motion sensor camera traps deployed at each site, providing continuous data on herbivore presence. Additionally, herbivore use was quantified by conducting dung surveys along four 8 m wide belt transects (two 150m and two 50m long) per site, with dung piles identified, counted, and their distance from the boma center noted. Data collection took place between April and October 2024. To analyze plant and herbivore community composition, we are conducting nonmetric multidimensional scaling (**NMDS**) and permutational multivariate analysis of variance (**per-MANOVA**). These methods will help identify patterns in vegetation structure and herbivore presence across the different boma treatments. Preliminary results indicate that boma sites occupied for 4–7 days have evolved into glades, attracting herbivores at rates comparable to those of 14–28-day sites. Moreover, we found no significant differences in vegetation cover or herbivore presence between the 14- and 28-day treatments, suggesting that extending boma occupancy beyond two weeks does not yield additional ecological benefits. These findings highlight the potential for shorter-duration bomas to serve as effective land restoration tools while maximizing the use of degraded areas in need of rehabilitation. Further analysis will refine our understanding of the relationship between occupancy duration, vegetation recovery, and wildlife attraction. This research provides valuable insights into sustainable boma grazing management and informs strategies for rangeland restoration. By optimizing boma placement and duration, livestock keepers can enhance ecosystem resilience, support biodiversity, and promote sustainable land use practices in semi-arid landscapes.

106 Significance of farm yard manure on small scale farmers in arids zone of Kajiado, Kenya

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Soils in Kenya are heavily depleted of essential nutrients due to generations of cultivation, especially in arid zones. Nutrient concentration in excreted cattle manure is relatively small (i.e., N < 1.7%; P < 0.6%). Current manure management practices led to low NCEs (average 27%) due to nutrient losses from excretion through storage and application. Analyses of soil samples from beneath the bomas (small enclosures) where animals are kept indicate that substantial losses of nutrients occur through leaching; ammonia volatilization and denitrification may also be involved. The current practice is to apply the manure at rates that appear to make poor use of this scarce resource. Farmers have few incentives to improve manure management given the small amounts of excreta and nutrients to be recycled. Yet, manure, both composted and fresh, represented the greatest N (16 kg ha⁻¹ season⁻¹) and C returns to the soil (312 kg C ha⁻¹ season⁻¹). Nutrient efficiency through improved farm yard manure application per unit area and recycling of nutrients can contribute to making low-input more productive and high-input systems less emissions intense. improve the efficiency of manure use. Further outputs of research to create tools to allow farmers to make better assessments of manure quality and match those with crop nutrient requirements and provide simple criteria to enable farmers to meet manure nutrient deficiencies with strategic use of inorganic fertilizers.

150 Assessment of manure management practices among livestock farmers in Uganda

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Livestock manure conventionally pollutes the environment and it may be turned into wealth through improved management. Survey research was conducted to determine existing manure management systems, their share in manure produced on farm and to quantify the extent of pollution through different systems. The commercial and subsistence farms of Uganda were selected for data collection using a pretested questionnaire through individual household visits. The extent of pollution by different system was calculated using IPCC Equation 10.23 and data were statistically analyzed using appropriate computer softwares. About 56.2 to 57.0% manure of large ruminants is kept in solid storage and 37.3 to 43.0% is used as burned fuel. Of the rest 4.80% is used in anaerobic digestion and 1.65% is lost as liquid slurry. The extent of manure used in solid storage was significantly ($p < 0.01$) higher in commercial than subsistence farms irrespective of areas. The extent of burned fuel between the two farm types differed and it was significantly ($p < 0.05$) higher in the commercial farm. The methane emission factor of dairy & other cattle (bulls & growing animals), small ruminants and poultry was calculated to be 6.77, 6.41, 5.42, 0.203 and 0.024 Kg CH₄/head/year, respectively and the annual emission was estimated to be 62.98, 92.3, 7.97, 5.89 and 7.62 Gg methane, respectively. Farmers' unawareness and their weak capacity and credit problem are the major constraints to improved manure management.

259 Post-feedlot manure emissions from Nellore steers cease after 35 days

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Feedlots produce large amounts of manure that undergo a series of reactions that emit greenhouse gases (**GHGs**). This study aimed to quantify the daily accumulation of methane (**CH₄**) and nitrous oxide (**N₂O**) emissions after the end of the feeding period of confined cattle consuming diets with different crude protein contents. The emissions were measured for 78 days immediately after a 107-day feedlot period with 40 Nellore steers at Embrapa Pecuária Sudeste, Sao Carlos, SP, Brazil. Treatments were two isoenergetic diets: one formulated with conventional ingredients (Conventional) and another with maximum inclusion of coproducts from ethanol production (Canapec). The feedlot facility consisted of four 400 m² dirt-surface pens, with 10 animals each (n=20/ treatment). The two dietary treatments consisted (on dry matter basis): Canapec diet with 17% crude protein (**CP**) (13.50% of sugarcane bagasse, 52.77% of corn grain, 30.0% of dry distillers' grains with solubles - **DDGS**, 0.66% of peanut oil, 0.51% of potassium chloride, 1.0% of calcite, 0.56% of urea, 1.0% of mineral mixture) and Conventional diet with 15% CP (25.50% of corn silage, 32.74% of corn grain, 35.00% of soybean hulls, 4.75% of soybean meal, 1.00% of urea, 1.00% of mineral mixture). Fecal samples collected from the rectal ampulla were analyzed for nitrogen (**N**) and CP content. GHGs were determined by static chambers that were randomly placed along three transects in the pens (n= 12/treatment), plus four static chambers outside the feedlot (1/pen) to measure soil GHG emissions (Blank). The chambers were closed and air samples were collected at 0, 30 and 60 minutes using a 50 ml polypropylene syringe, then transferred to a pre-evacuated chromatography vial (10 ml). The samples were analyzed by gas chromatography, and fluxes were determined linearly due to gas accumulation inside the chambers within the one-hour collection period, considering temperatures inside and outside the chambers to correct gas flux calculations. These values were multiplied by 24 to estimate daily fluxes and integrated to calculate cumulative emissions (mg m⁻²). Emissions were monitored across eight sampling day(s) after the end of the feeding period: 0, 1, 5, 12, 19, 34, 48, and 77; although the last two dates had no measurable emissions of GHG and the values for day 34 were near zero. Mean temperature and relative humidity were 23.6°C and 80.9% with 456 mm total rainfall, 70% occurring in the first 35 days. The means of the 34 days with measurable data were subjected to analysis of variance using the PROC MIXED of SAS. There were significant differences in N and CP (P=<.0001) levels for Conventional and Canapec, with values of 2.5 and 1.9%, and 15.8 and 12.4%, respectively. However, no statistical difference was observed in CH₄ (P=0.4841) and N₂O (P=0.7477) emissions: 13.9 and 7.3 CH₄ – C mg m⁻², and 14.7 and 11.5 N₂O – N mg m⁻², for Conventional and Canapec, respectively. Therefore, animals fed the Canapec diet were more digestively efficient, excreting less N and CP with the potential for lower GHG emissions from manure, which ceased approximately 35 days after confinement.

365 Estimating greenhouse gas emission reduction from livestock manure management through biodigesters and thermal biogas application

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Anaerobic digestion of livestock manure produces biogas and organic fertilizer, supporting waste management, renewable energy, and greenhouse gas (GHG) emission reduction. With growing government commitment to household biogas adoption in sub-Saharan Africa (SSA), accurate quantification of GHG reductions is vital for national inventories and carbon crediting. The IPCC Tier 2 method, which improves accuracy over the Tier 1 approach commonly used in SSA, was applied in this study to estimate GHG reductions from manure managed through biodigesters and thermal biogas use. Data were collected from 329 biodigester-user households and 307 non-user households with similar socioeconomic status in Ethiopia and Kenya, using pre-tested questionnaires and simple field tools via digital ODK. Socio-demographic data were analyzed descriptively, and GHG emissions were estimated using a customized Excel-based calculator following the IPCC Tier 2 methodology. Baseline emissions were derived from non-user data and included the following emission sources: methane (CH₄) emissions from manure management, carbon dioxide (CO₂) emissions from biomass (firewood and charcoal) and fossil fuels (kerosene and liquefied petroleum gas). Project emissions were derived from biodigester user data and included: CH₄ from manure untreated in the biodigester, CO₂ from biomass and fossil fuel stacking (users relying on additional fuels besides biogas), CH₄ leakage (default 10% of total methane produced), biogas stove efficiency (90%) and CH₄ emissions from digestate storage. In Kenya, CO₂ emissions from biomass were calculated using two non-renewable biomass (fNRB) values (45% and 92%), pending approval by the Supervisory Body of the Article 6.4 mechanism. The reductions in emissions were calculated by subtracting project emissions from baseline emissions. Statistical significance was assessed at $p < 0.05$. Findings showed firewood remains the dominant household energy source. Digestate use reduced chemical fertilizer application by 43.93 kg/year in Ethiopia and 36.58 kg/year in Kenya. In Ethiopia, all users installed fixed-dome biodigesters, with 90.7% having a capacity of 6 m³. In Kenya, 49% used fixed-dome and 51% used premanufactured digesters, with 82.4% having a capacity of 8 m³ or larger. In Kenya, baseline emissions were 3.30 and 6.40 tCO₂e/year/household for fNRB values of 45% and 92%, respectively, while project emissions were 1.59 and 3.09 tCO₂e/year/household, respectively – resulting in reductions of 1.71 and 3.30 tCO₂e/year/household. Ethiopia's emission reduction results will be finalized using the same approach and shared during the conference. Biodigester adoption significantly reduced the use of traditional polluting fuels and chemical fertilizers. Applying the IPCC Tier 2 method enhanced the accuracy of estimating emissions reductions. The digital ODK survey tool, data collection protocol, and emissions calculation methodology – combined with a user-friendly Excel tool – offer a simplified, transparent, and harmonized approach for quantifying GHG reductions in smallholder systems.

377 Greenhouse gas emissions from manure heaps of dairy cattle fed diets with different feed qualities

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Manure is a dominant source of livestock-related methane and nitrous oxide emissions. Methane in manure is produced anaerobically by methanogenic archaea, while nitrous oxide is generated through nutrient-dependent nitrification and denitrification processes, with the production of these greenhouse gases being directly influenced by diet quality consumed by livestock. In East African smallholder dairy systems, feeds are often low in nitrogen, which limits manure nitrogen excretion and thus nitrous oxide emissions. Efforts being made to improve diet quality to enhance milk production and overall productivity may unintentionally increase manure emissions. The present study measured greenhouse gas (GHG) emissions from uncovered solid manure heaps, a common manure management system found in smallholder dairy systems. The manure was collected from dairy cattle in a feeding trial where the animals were provided three diets representative of local diets: low-quality (crude protein [CP]: 10%, digestible energy [DE]: 59%), medium-quality (CP: 12%, DE: 61%), and high-quality diets (CP: 14%, DE: 63%). Animals were housed individually, and manure (mix of dung and urine) was collected over three consecutive days by scraping manure from the floor (no bedding was used). Manure was heaped into concrete troughs (n=3, ca. 100 kg fresh weight per trough) and was left uncovered to simulate solid heaping over four months. Each heap measured ca. 0.8×0.7×0.7 m. To measure GHG emissions, troughs were covered with air-tight covers and headspace air samples were collected for GC analysis after 0, 4-, 8-, 12- and 16-minutes using syringes. Gas sampling was conducted daily for one month and then 3x per week for an additional three months. In addition, manure chemistry was measured, and the faecal-N: urinary-N ratio (the main determinant of manure N₂O emissions) of manure was determined. The trial is ongoing and will run until April 2025; therefore, no results are given here but will be presented at the conference. Once complete, this study will contribute to the understanding of how dietary modifications in semi-intensive dairy systems can influence environmental outcomes, particularly manure GHG emissions.

394 Post-thermophilic application of *Tithonia diversifolia* enhances compost nutrient levels and contributes limited greenhouse gas emissions

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Composting is a sustainable method for enhancing nutrient availability and soil fertility in agricultural systems, although it is also associated with greenhouse gas (GHG) emissions. The incorporation of specific inputs may influence GHG fluxes. Despite the environmental implications of composting, limited data exists on GHG emissions and nutrient dynamics in small-scale household farms, particularly in sub-Saharan Africa (SSA). This study evaluated the effects of various organic inputs on compost nutrient content and GHG emissions. The treatments included: (i) cattle manure + 100% biochar + shade-dried *Tithonia* at day 0 (CMBT), (ii) cattle manure alone (CM), (iii) cattle manure + 100% biochar + *Tithonia* at day 70 (CMBT70), and (iv) cattle manure + fresh *Tithonia* + dry maize stalks (conventional) replicated thrice. Compost samples were collected at different composting stages and analyzed for total nitrogen (N), phosphorus (P), potassium (K), and carbon (C) using standard analytical methods. GHG fluxes (CO₂, CH₄, N₂O) were measured using a circular vented static chamber and quantified via SRI 8610C gas chromatography. Data analysis was performed using R ('agricolae' package). The CMBT resulted in the highest nitrogen content (2.87%), while CM and CMBT70 showed higher total carbon and lower C:N ratios. Peak GHG emissions occurred during the thermophilic and curing phases, primarily driven by moisture and temperature. The CMBT treatment produced the highest emissions of CH₄ (p=0.01), CO₂ (p=0.03), and N₂O (p<0.001). Comparing to the conventional practice, CMBT increased CO₂ (19%), CH₄ (62%), and N₂O (95%), whereas CMBT70 decreased CO₂ (13%), CH₄ (2%), and N₂O (12%). Therefore, CMBT70 enhanced nutrient quality while minimizing GHG emissions. Further studies should explore microbial dynamics to explain their role in compost quality and emissions.

440 Current manure management in Uganda and Ethiopia affects manure quality, greenhouse gas emissions, and health.

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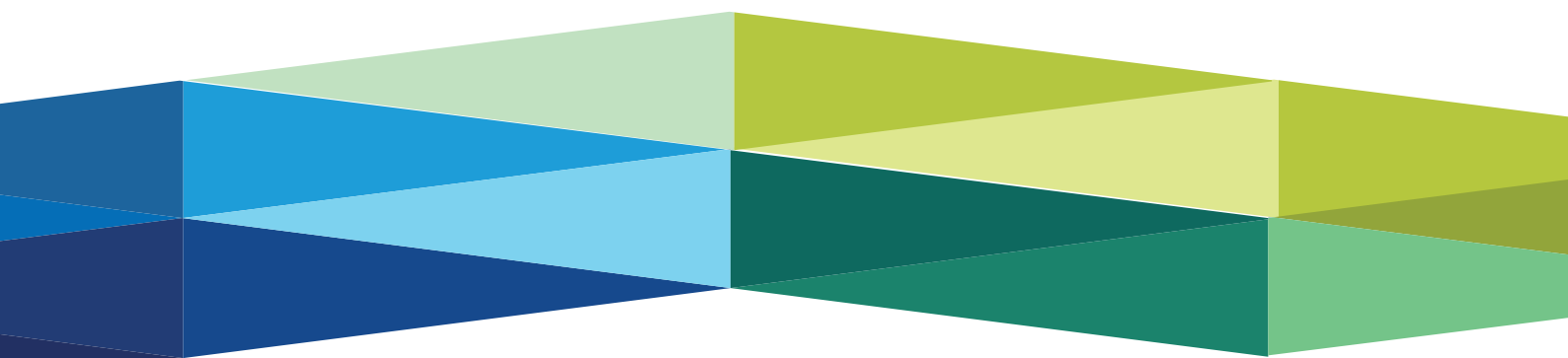
Livestock populations in developing economies are increasing, and with it the amount of manure generated on farms. If handled well, manure is a good soil amendment to address the serious constraint of crop production in sub-Saharan Africa, where farmers do not meet recommended fertilizer rates. However, mismanagement of manure results in nutrient loss, air and water pollution, health risks from zoonotic diseases, and greenhouse gas (GHG) emissions. So far, few coherent studies on manure management in SSA exist, and the contribution of different manure management systems to GHG budgets is unclear. To address this data gap and as a baseline for the development for context-specific manure interventions, we carried out household surveys in mixed crop-livestock systems in Uganda and Ethiopia to assess current manure management practices, constraints, and drivers of improved manure management practices. We found that most farmers in crop-livestock systems use manure as fertilizer in Uganda (92% of pig farmers, 88% of cattle and small ruminant farmers) and Ethiopia (95% of cattle and small ruminant farmers). In both countries, over 96% of the manure storage facilities have neither cover nor floor, exposing manure to rain and direct sunlight, which reduces the nutrient content and leads to high ammonia emissions as well. Heaping is a predominant manure storage method (85.5% farmers in Ethiopia, and 86% pig farmers in Uganda), which can potentially result in high methane emissions. In addition, we found that safety precautions against zoonotic diseases are rarely done, use of gloves, overalls and masks is low (below 7%) among the farmers. The current manure management practices can therefore potentially lead to environmental low manure fertilizer quality, pollution, and health risks. Farmers indicated that reasons for sub-optimal manure management across the countries and value chains are the lack of information, limited labor, and limited capital to invest in better manure management practices. On the other hand, we observed that better fertilizer quality, and improved health of animals and humans could motivate farmers to improve their manure management. Consequently, there is a need to develop and promote tailored win-win manure management practices that are feasible for smallholder farmers to help them address food security, soil fertility, health issues, and climate change mitigation and adaptation.



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Theme 5: Greenhouse gas emissions from agricultural soils and grazing systems



10 Impact of agricultural land use practices on GHG emissions in sub-Saharan Africa: a DOLS approach

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Sub-Saharan Africa's agricultural sector plays a crucial role in economic development but also contributes significantly to greenhouse gas (**GHG**) emissions. This study investigates the impact of various agricultural practices on GHG emissions from 1970 to 2023 using a Dynamic Ordinary Least Squares (**DOLS**) approach, performed in Stata. The dependent variable is GHG emissions, while the independent variables include agricultural land, farm buildings, cropping land, permanent meadow and pasture, permanent meadow cultivated, arable land, permanent natural pasture, permanent crops, and temporary crops. Data for these variables were collected from FAOSTAT and World Bank databases, ensuring consistency and regional comparability. The results show that arable land and permanent crops significantly ($p < 0.05$) increase GHG emissions, driven by intensive farming and land conversion. Farm buildings and cropping land also have a positive association with emissions, reflecting the industrialization of agriculture. Conversely, permanent natural pastures have a neutral or slightly negative effect on emissions, indicating that natural grazing areas contribute less to emissions compared to cultivated land. The findings emphasize the critical role of land use in GHG emissions across Sub-Saharan Africa. Agricultural intensification, particularly in arable land and permanent crop production, is a key contributor to rising emissions, while natural pastures offer a more sustainable land-use alternative with a lower environmental impact. To reduce GHG emissions, policies should focus on promoting sustainable land management practices such as conservation tillage, agroforestry, and organic farming. Encouraging greater use of natural pastures and implementing rotational grazing systems can also reduce emissions while ensuring agricultural productivity. Moreover, addressing potential conflicts between crop and livestock farmers over pasture use requires coordinated land-use strategies. Further research should explore the role of fertilizer use, irrigation intensity, livestock density, and mechanization in driving GHG emissions for a more comprehensive understanding.

16 GHG emissions from agricultural soils and grazing systems in Kenya's pastoralist regions

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Greenhouse gas (**GHG**) emissions from agricultural soils and grazing systems represent a critical component of climate change dynamics, particularly in Kenya's pastoralist regions. Pastoralist communities in Kenya predominantly rely on livestock for their livelihoods, resulting in unique interactions between agricultural practices and GHG emissions. This study examines the sources and magnitude of GHG emissions within these ecosystems, characterized by traditional livestock rearing and extensive land use practices. By integrating field measurements, survey data, and secondary sources, the research identifies key drivers of emissions and evaluates potential mitigation strategies. Findings suggest that improved land management practices, such as agroforestry and rotational grazing, could enhance carbon sequestration and reduce overall emissions. These insights contribute to a broader understanding of sustainable pastoralism in the face of climate change challenges.

This research collected data from various pastoralist communities in Narok and Kajiado Counties in Kenya to assess the extent and sources of GHG emissions. Soil samples and gas flux data were gathered through field measurements, while surveys and interviews with pastoralists provided insights into land management practices. Additionally, secondary data from climate reports and agricultural studies were analyzed to estimate emissions linked to grazing activities. The primary focus was on carbon dioxide (**CO₂**), methane (**CH₄**), and nitrous oxide (**N₂O**) emissions, measured using gas chromatography and remote sensing techniques.

Key findings indicate that overgrazing and inadequate soil management contribute to soil compaction and degradation, leading to reduced soil organic carbon levels and increased emissions. Limited use of chemical fertilizers still results in measurable N₂O emissions, further exacerbating the GHG footprint of pastoralist systems. The study also highlights the role of traditional pastoralist practices, showing that while nomadic grazing can cause localized overgrazing, rotational grazing methods enhance soil health and sequester carbon. Furthermore, climate variability influences emissions, as shifting rainfall patterns and rising temperatures impact pasture quality, livestock movement, and grazing intensity. These factors either amplify or mitigate emissions depending on the region and season. The findings suggest that implementing improved land management techniques – such as agroforestry and controlled grazing – could enhance carbon sequestration and reduce overall emissions in pastoralist areas.

GHG emissions in Kenya's pastoralist regions are driven by a combination of land-use practices, climate variability, and traditional livestock management methods. By integrating sustainable grazing strategies with local knowledge, it is possible to develop resilient pastoral systems that contribute to climate change mitigation. Future research should focus on refining emission measurement techniques and evaluating the effectiveness of specific interventions to support sustainable pastoralism in the face of on going environmental challenges.

19 Climate-smart dairy farming for GHG mitigation and food security in the Salale highlands, Ethiopia

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Global livestock productions in general and the dairy value chain in particular are at the crossroads of improving productivity and reducing environmental footprints to meet the growing demand for livestock products under a changing climate condition. Smallholder dairy farms must undergo a profound transformation in order to increase productivity, mitigate or reduce greenhouse gas emissions, and contribute to food security. The overall objective of this study was to investigate the economic and environmental benefits of climate-smart dairy farming practices, with particular emphasis on 480 smallholder farmers selected from three farming systems in the Salale milk belt, central Ethiopia. The present study adopts a mixed research design with data from household surveys, key informant interviews, focus group discussions, livestock activity, and biological data. We employed the econometrics model and a cradle to farm gate lifecycle assessment approach. The result showed that resource constraints, supply chain bottlenecks and market linkages, inadequate services, and a scarcity of information on dairy management have been reported as hindering improved dairy technology adoption and implementation in the study area. The adoption of a few or the integrated package of improved practices had a significant impact on farmers' food security. Enteric fermentation was the primary source of GHG emissions, and methane emissions from enteric fermentation and manure management accounted for the majority of total emissions across farms. The estimated average carbon footprints vary depending on farming systems, Global Warming Potential (**GWP**), and allocation methods used. The overall average carbon footprints of milk production were 1.91 and 2.35 kg CO₂e/kg fat and protein-corrected milk (**FPCM**) for different IPCC GWPs, and the estimated carbon footprints showed significant variation across farming systems. Our findings showed that using improved dairy farming practices would increase animal productivity, reduce GHG emissions, and enhance farmers' food security. Interventions that overcome the constraints related to access to farm resources and the supply chain for improved practice inputs, services, and output markets could facilitate the uptake of dairy technologies. We recommend increasing the proportion of improved forage as well as chemically upgrading straw and crop residue to enhance feed digestibility and efficiency, thereby increasing milk yield and reducing carbon footprints of milk production.

20 Impact of conservation agriculture on soil greenhouse gas emissions in maize systems in Buea, Cameroon

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As global efforts intensify to address climate change and enhance agricultural sustainability, conservation agriculture emerges as a key strategy to reduce the carbon footprint of farming. Despite its promise, there is limited understanding of how conservation practices influence greenhouse gas (**GHG**) emissions in sub-Saharan Africa, including Cameroon. This study examines the effects of conservation agricultural practices—specifically reduced tillage and organic fertilization—on soil GHGs (CO₂, N₂O, and CH₄) emissions over two growing seasons (2020 and 2021). Conducted at the University of Buea in Cameroon, the study employed a split-plot design to compare zero tillage (**No-Till**) with conventional tillage (**Till**) and fertilizer treatments: no fertilizer (**CON**), synthetic fertilizer (urea) (**SYN**), and organic fertilizer (composted municipal solid waste) (**ORG**) in six combination treatments. The hybrid maize cultivar CMS 8704 was used as the test crop. GHG emissions were measured using the static flux chamber method, and flux rates were calculated with the HMR package in R. The results show that in the first growing season, cumulative N₂O efflux was in the following order: NoTill+SYN > Till+ORG > NoTill+CON > Till+CON > Till+SYN > NoTill+ORG, while cumulative CO₂ emission was in the order: Till+ORG > Till+SYN > Till+CON > NoTill+CON > NoTill+ORG > NoTill+SYN. CH₄ emission was in the following order: Till+CON > Till+ORG > NoTill+SYN > Till+SYN > NoTill+CON > NoTill+ORG, and efflux values were negative, indicating that the upland soils of the study area consumed methane. Tillage practice and fertilizer types significantly influenced ($p < 0.05$) GHG emission in 2020. In the 2021 study season, N₂O was emitted in the following order: Till+SYN > NoTill+SYN > NoTill+ORG > Till+CON > Till+ORG > NoTill+CON, while CO₂ was emitted in the following order: Till+ORG > NoTill+SYN > Till+CON > Till+SYN > NoTill+ORG > NoTill+CON. Although NoTill+ORG recorded positive CH₄ values, most plots still recorded negative CH₄, indicating that most of the plots acted as sinks for methane in 2021. Soil moisture and temperature did not significantly affect ($p > 0.05$) GHG emissions in both seasons. The study concludes that reduced tillage combined with organic fertilization can effectively reduce GHG emissions in agricultural soils. It recommends further research on the impacts of different fertilizer application rates and combinations over longer periods to better understand their effects on GHG emissions.

23 Grazing affects soil organic carbon stock directly and indirectly through herbaceous species diversity

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The impact of livestock grazing on soil organic carbon (**SOC**) stocks in the Sahel has been poorly documented due to a lack of data from different grazing intensities. This study evaluated SOC stocks under four grazing intensities within 0-30 cm soil depth in ry savanna ecosystems of Senegal. It also examined possible indirect relationships between grazing and SOC, through herbaceous species diversity, herbaceous biomass, and carbon-nitrogen ratio. Grazing intensity was inferred from proximity to settlements, with closer areas experiencing more frequent and intense grazing. Four sites were selected: (i) a settlement site with year-round grazing (high grazing), (ii) a site ~10 km away with rotational grazing (moderate grazing), (iii) a dry-season grazing enclosure (light grazing), and (iv) an 18-year-old fenced area (no grazing). Light- and no-grazing sites were established near the moderate- and high-grazing areas, respectively, to ensure similar soil type, topography, and microclimate across treatments. Therefore, observed differences were attributed mainly to grazing intensity and its indirect effects on vegetation. Transect survey methods were used to sample soil and vegetation data within each site. Mixed-effects models were used to analyze the impact of grazing on SOC stocks, with a plot as a random factor. Linear models assessed the relationships between grazing intensity, herbaceous species diversity, biomass, and SOC. Piecewise structural equation modelling (**pSEM**) was employed to explore potential indirect effects of grazing through diversity and biomass, treating grazing intensity as an ordered categorical variable. SOC stocks significantly differed among the four grazing intensities, and higher stocks were observed with increased intensity. Herbaceous diversity interacted significantly with grazing intensity in influencing SOC, while biomass had no significant effect. Increased grazing intensity also reduced the soil C:N ratio by decreasing species diversity. In turn, the altered C:N ratio positively influenced SOC accumulation. In conclusion, this study found that increased grazing intensity promoted SOC stocks both directly and indirectly through herbaceous species diversity.

53 Estimating the integral value of climate change mitigation in livestock farming

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Socio-ecological systems are conceptualized as interconnected networks where human societies are deeply integrated within the dynamics of natural ecosystems. These societies must adapt, prosper, and evolve within the ecological constraints of the planet. Natural systems provide ecosystem services essential for human well-being, while social systems interact with these natural systems, producing both beneficial environmental outcomes and adverse externalities. Among economic activities reliant on ecosystems, livestock production stands out, alongside crop farming, as a vital livelihood strategy. It not only relies on natural ecosystems but also contributes ecosystem services and environmental benefits. Livestock systems encompass all aspects related to the production, utilization, and trade of livestock and their products. This includes the spatial distribution of livestock, production systems employed, current and projected trends in production and consumption, the individuals involved in livestock farming, and the associated benefits and environmental impacts. One notable ecosystem service within livestock systems is carbon storage and sequestration, facilitated through cultivated forages, rangelands, and agro-silvopastoral systems. However, human activities, particularly consumption patterns, exert significant pressure on natural ecosystems. To counteract this, policies, institutional frameworks, and technological innovations are being developed to mitigate methane emissions from ruminants. These interventions hold substantial promise for promoting the sustainability of both natural and social systems. By integrating carbon sequestration and methane reduction strategies, livestock systems can achieve a more favorable carbon balance, thereby mitigating their contribution to climate change. Although there is evidence supporting the technical effectiveness of these measures, critical gaps remain regarding the costs of inaction, the investments required for implementation, their large-scale economic viability, and broader impacts. These uncertainties hinder the promotion and financing of such initiatives. This study aims to establish a feasible framework for assessing the economic and social value of interventions that enhance carbon sequestration and reduce methane emissions in livestock systems. The economic valuation employs market-based pricing methods, drawing on carbon prices from major global emissions trading systems and the regulatory instruments used in the target country. Social valuation involves stakeholders' awareness and perceptions of carbon storage, sequestration, and methane mitigation. Through contingent valuation methods, including Discrete Choice Experiments and Public Goods Games, we estimate their willingness to financially support these mitigation strategies. We have applied this approach in three case studies in Colombia, focusing on reducing methane emissions through silvopastoral systems with improved pastures and minimizing the carbon footprint of meat and milk production. We found that the economic value of this environmental benefit ranges from US\$ 6 to US\$ 9 per animal in beef production, and US\$ 58 per animal in dairy production. Currently, we are applying this method to evaluate mitigation strategies on dairy farms in Kenya, we hope to present our result in 2025. These findings indicate that the environmental benefits generated or preserved through these interventions exceed the implementation costs, demonstrating their potential for promoting sustainable practices in livestock systems.

58 Methane emission and blood total serum levels of different breeds of cattle grazing in Ghana

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Methane (**CH₄**) is a major greenhouse gas primarily produced by ruminants with cattle being a major emitter through enteric fermentation and manure production. Grazing by cattle constitutes one of the significant activities that emit large volumes of methane. Livestock farming is known to generate greenhouse gas emissions, specifically methane, which contributes approximately 14.5% of global anthropogenic emissions. A study was conducted at the Livestock and Poultry Research Centre (**LIPREC**), University of Ghana, Legon to investigate methane emissions and total blood serum concentration (**TBSC**) from cattle grazing in the Coastal Savannah zone of Ghana and the effect of temperature and humidity on methane emission. The study also evaluated the chemical composition of forages consumed by cattle. Twenty cattle, comprising of four breeds (Sanga, West African Shorthorn [**WASH**], Sanga Cross and Sokoto Gudali) were used. Five cattle from each breed with an average weight of 125 ± 2.4 kg were used. Methane emission, temperature and humidity were measured monthly from August 2023 to April 2024 when animals were ruminating using the handheld gas methane detector (GASTiger 2000, Stark Instrument Ltd, China). Measurements of enteric methane were taken on individual animals in the wet and dry seasons. The handheld gas methane detector was placed close to the nose of the cattle where the highest concentration of methane was likely to be emitted to measure enteric methane. This method of measuring methane may not be as accurate as the respiratory chamber method. Enteric methane was measured after grazing. Methane emissions from manure were measured from the paddocks when the cattle were released for grazing. Blood samples (5ml) were taken from the jugular vein into gel and clot activator tubes. TBSC was assessed using a handheld refractometer. Data obtained were analyzed in a randomized complete block design and subjected to ANOVA using GenStat version 12.1. The Student-Neuman-Keuls' test was used to determine level of significance. Some common forages grazed by cattle were *Megathyrus maximus*, *Cenchrus ciliaris*, *Cynodon nlemfuensis*, *Sporobolus pyramidalis*, *Stylosanthes sp.*, *Gliricidia sepium*, *Albizzia lebbek*, *Milletia thonningii* and *Leucaena leucocephala*. The wet season had better nutritional quality of forages grazed than dry season. Results indicated significant differences ($p < 0.05$) in methane emissions between the breeds, with the WASH emitting the lowest and the Sanga breed the highest. Seasonal variations were observed, with higher emissions during the dry season. Methane emission from manure was higher in the wet season (834 ppm) than in the dry season (580 ppm). Low temperatures and high humidity favoured high methane production and vice versa. Total blood serum concentration was higher ($p < 0.05$) during the wet season than in the dry season for all the cattle breeds. However, TBSC from Sanga cattle was the highest during the wet season and the lowest level in the dry season. Sokoto Gudali, Sanga and WASH recorded TBSC below the normal range (5.7-8.1 g/dl) for healthy cattle in the dry season. It is recommended that more effort should be made to mitigate methane emission when temperatures are low and humidity is high.

60 Are Kenyan dairy farmers worried about climate change? A social valuation experiment

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Climate change and livestock farming are closely interconnected, as livestock production, particularly ruminants, significantly contributes to greenhouse gas emissions, such as methane. In Africa, dairy farming faces unique challenges due to rising temperatures, precipitation variability, and pasture degradation, all of which impact productivity and sustainability. High temperatures reduce reproductive efficiency and milk production in cows, while small-scale production systems are more vulnerable to climate risks. However, implementing resilience strategies, such as hybrid and improved forages, silvopastoral systems, and enhancing manure management, can help mitigate these impacts and ensure more sustainable production on the continent. Dairy farmers in Kenya contribute to climate change while also facing the consequences of it on their farms. Our goal is to determine if Kenyan dairy farmers perceive the climate change effects, are worried about them, and are willing to implement mitigation strategies on their farms. To achieve this, we developed a social valuation experiment with 46 dairy farmers from the Nandi and Uasin Gishu counties. Our experiment consists of the estimation of a social value indicator composed of three sub-indicators, knowledge, perception, and willingness to action. Knowledge refers to the information that dairy farmers have about climate change, perception shows whether dairy farmers are worried about the consequences of climate change on their lives, and willingness to action answers the question of whether they are willing to implement mitigation strategies on their farms. Social valuation is one of the three components of the integral valuation of ecosystem services and environmental benefits. The information for the experiment was collected through surveys in July 2024. For the design of the social value indicator and its sub-indicators, we implemented the Principal Components Analysis method. The social value indicator ranges from 0 to 1. Values close to 0 show low social value, and values close to 1 show high social value. We estimate a social value of climate change mitigation strategies of 0.72 (high). Mainly explained by high values of knowledge (0.93) and perception (0.81). Although dairy farmers are worried about climate change, their willingness to action is low (0.38). The main reason they mention for explaining this is that they do not have the necessary resources to implement mitigation strategies on their farms. We also consulted on the mitigation strategies they would like to implement and more than 50% of those surveyed are interested in adopting hybrid and improved forages as a strategy to reduce emissions. We conclude that dairy farmers are worried about climate change, they would like to mitigate emissions, but they need support from other actors since they cannot finance the resources necessary for mitigation on farms.

70 Effectiveness of innovative conservation agriculture in addressing climate shocks and sustainable resilience building in Ethiopia

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Recent studies indicate that 85% of potential agricultural lands in Sub Saharan Africa are nutrient degraded. Improving food security and environmental conservation should be the main targets of innovative farming systems. This paper discusses the applicability and potential benefits of conservation agriculture Innovation under the unfolding climate change scenario in the dry land farming system of Ethiopia. Both qualitative and quantitative methods of data collection were implemented. A cross-sectional household survey was collected from a multi-stage sample of 420 smallholder mixed farmers selected from three gynecology of the lower Omo River Basin dry land farming districts in Southern Ethiopia and systematically analyzed. Key informant interviews were held with policy makers and agricultural extension professionals. Three checklist based focus group discussions were held with farmers from selected villages from different agro-ecology. In high rainfall areas, the soils are susceptible to soil erosion and face fertility decline while in low rainfall areas (Mancha, Offa District) crops fail due to sub-optimal water use efficiency due to poor dry land farming practices. Current agricultural development policies, strategies, and technologies are not in sync with the global effort to mitigate current and future climate change shocks mitigation measures, nor do they provide a window of opportunity for smallholder farmers to build climate resilience. Innovative Conservation agriculture (**ICA**), based on minimum tillage, crop residue retention and crop rotations has become a proposed solution against climate shocks mitigation, poor agricultural productivity, farming practices in dry land agriculture and soil degradation simultaneously enhancing food security, *climate* adaptation, and reduce GHG emissions. The result show application of integrated ICA solutions using improved seed variety, compost, row planting, soil covering and agroforestry, provide farmers with the benefits of enhanced food security, sustainable fodder feeds production for livestock and climate change adaptation in dry land farming by reducing GHG emissions from farm plots. ICA addresses multiple challenges: climate shock mitigation, low agricultural productivity, unsustainable farming practices in dryland areas, and soil degradation. Simultaneously, it enhances food security, climate adaptation, and GHG The study revealed that ICA reduce soil loss from 35.5 to 14.5 t/ha/year, have 50-70% greater infiltration and increase 42% of organic carbon. Long term analysis using Agricultural Production System Simulator showed that ICA can increase yield from 3.6 to 4.4t/ha in areas having >770 mm. Mulching, manure/dung application, composting and Crop rotation provides farmers with enhanced food security and reduced livelihood vulnerability strengthening adaptability to climate shocks in dry land mixed farming areas of Ethiopia (both crop production and animal husbandry). Therefore, ICA as a key component of Climate-smart agriculture innovations are essential for enhancing household food security, reducing vulnerability to climate shocks, and reducing farm-level GHG emissions in the face of climate change for a sustainable agricultural system. The study also revealed that dry land farming areas that practice livestock production experience much soil fertility and reduced soil degradation. The researchers recommend that the livestock production system has to be integrated in to agricultural systems to address climate shocks and reduced GHG emissions.

77 Greenhouse gas emissions under different land uses in mixed crop-livestock systems in sub-humid Zimbabwe

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Mixed-crop livestock systems are an important component in the livelihoods of many people in Africa. However, the sector is perceived to contribute to greenhouse gases (**GHGs**), such as nitrous oxide (**N₂O**), methane (**CH₄**) and carbon dioxide (**CO₂**) which causes climate change. Accurate measurements GHGs emissions are essential in the global effort to mitigate climate change. In our study we quantified GHGs emissions from different land uses in mixed crop-livestock systems in sub-humid Zimbabwe. The greenhouse gas measurements were done under four common land uses which are croplands, grazing lands, cattle kraals and livestock congregation areas under high- and low-density crop-livestock systems. The high-density crop-livestock were the commercial farms and the low density were smallholder farms. The measurements were done between November 2023 - October 2024. Greenhouse gas measurements were done using the static chamber method, replicated four times. In croplands, static chambers were put in the maize row and between maize interrow. GHGs were analyzed by gas chromatography. During each GHG sampling, soil moisture and mineral nitrogen were measured in the top 0–20 cm soil depth. Data analysis was done using R software, version 4.4.1 (R Core Team, 2024). There was a significant ($p < 0.001$) difference in daily N₂O and CH₄ between the land uses and across sites. Mean 400µg N₂O-N m² hr⁻¹ were found in cattle kraals at commercial. We recorded average daily emissions of < 50µg N₂O-N m² hr⁻¹ in grazing lands across the two sites. Cumulative annual emissions of CH₄ and N₂O varied significantly ($p < 0.001$) across land uses and across the two sites. Significant high N₂O emissions were observed in cattle kraals and livestock congregation areas. Furthermore, inorganic nitrogen fertilizers depicted high N₂O emissions as high emissions were observed two days after mineral N application in maize croplands. At commercial site, significant emissions of 4500kg CH₄-C ha⁻¹ were observed from cattle kraals, and in smallholder systems daily average methane emissions of 250kg CH₄-C ha⁻¹ were found in cattle kraals. N₂O and CH₄ emissions were significantly higher in cattle kraals with emissions several folds of magnitude higher than other land uses. A positive correlation was found between soil moisture and daily N₂O emissions. Highest N₂O emissions were observed at water-filled pore space of 55–70%. Smallholder croplands were net sinks for CH₄ whilst commercial croplands emitted significant CH₄ emissions. Based on the results soil mineral N and soil moisture were key sources of greenhouse gas emissions. The study showed that smallholder croplands contribute to low amounts of GHGs emissions and grazing lands were net CH₄ sinks. Our study showed that cattle kraals and livestock congregation areas are potential hotspots for GHGs emissions and these sources are often neglected in global GHGs inventories. Therefore, the findings are pivotal to policymakers in developing low-carbon development under different land uses in African agricultural systems. In addition, grazing lands are considered to contribute significantly to low emissions as they are carbon sinks that can help in climate change mitigation.

78 Carbon accrual in contrasting beef cattle grazing systems

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Anthropogenic activity is increasing the emissions of greenhouse gases such as nitrous oxide, methane, and carbon (C) dioxide, resulting in climate change. Grasslands are a crucial global ecosystem, serving as the foundation for livestock production and being one of the largest terrestrial C sinks. Including N-fixing legumes in grass-based monoculture systems is an alternative to enhance sustainability and C accrual. We evaluated the 9-year effects of legume inclusion and nitrogen (N) fertilization on C dynamics in three layers (0-15, 15-30, and 30-90 cm). Treatments were established in 2014 and included three grazing systems: 1) Grass-N: a mixture of oat-ryegrass during the cool season and bahiagrass (*Paspalum notatum* Flüggé) during the warm season, with 112 kg N ha⁻¹ applied each season (224 kg N ha⁻¹ yr⁻¹); 2) Grass-CL: oat and ryegrass during the cool season plus a blend of clovers (crimson, red, and ball clovers) with 34 kg N ha⁻¹ whereas during the warm season was a bahiagrass monoculture with no N fertilization; 3) Grass-RP: the cool season was a similar treatment than the previous, and during the warm season was a bahiagrass-rhizoma peanut (*Arachis glabrata* Benth) mixture, with no N fertilization. Each pasture was continuously stocked with two steers and variable stocking rates; this rate was greater for Grass-N than for the other treatments. Soil samples were taken in 2014, before planting the perennial species, and resampled in 2023. Response variables included C and N concentrations, $\delta^{13}\text{C}$, soil organic C (SOC), and total N. Cumulative SOC and total N were obtained by summing up the three soil layers within each experimental unit. Variables were adjusted by using the equivalent soil mass (ESM). Soil C concentration presented a depth effect ($P = 0.036$), with greater values in the top layer, averaging 6.65, 4.74, and 2.57 g kg⁻¹, respectively. Similarly, N concentration presented a depth effect ($P = 0.025$), being greater for the top layer compared to the second and third, averaging 0.75, 0.48, and 0.26 g kg⁻¹, respectively. Soil $\delta^{13}\text{C}$ decreased with depth ($P < .001$), averaging -19.6, -20.4, and -21.3‰ for the first, second, and third layers. There was a depth effect on SOC stock ($P = 0.006$) and total N stock ($P = 0.001$). The SOC stock was greater for the third layer (32.5 Mg ha⁻¹) compared to the first (15.3 Mg ha⁻¹) and second (14.3 Mg ha⁻¹). Besides, greater total N was observed in the last layer (2.9 Mg ha⁻¹) compared to the first (1.7 Mg ha⁻¹) and second (1.4 Mg ha⁻¹) layers. There were no differences in cumulative SOC ($P = 0.93$) and total N ($P = 0.56$) among treatments. Cumulative SOC averaged 62 Mg ha⁻¹ (SE = 9.89 Mg ha⁻¹), and total N averaged 6.07 Mg ha⁻¹ (SE = 0.98 Mg ha⁻¹). Similar SOC and N stocks were possible by including legume species in grass-based grazing systems with a reduction of external N inputs. Soil C accrual is an important ecosystem service provided by grazing systems, with the potential to add economic value to the overall farm.

81 Atmospheric carbon dioxide removal *vis-a-vis* soil amelioration through enhanced rock weathering in Indian acidic soil

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Since the industrial era began, human activities have driven global warming through increased greenhouse gas emissions, particularly CO₂, which persists long-term in the atmosphere. Enhanced Rock Weathering (**ERW**) accelerates the natural weathering of silicate rocks to absorb atmospheric CO₂. However, studies assessing ERW's CO₂ removal (**CDR**) potential using indigenous basalt rock dust (**BRD**) in the Indian sub-tropics are limited. Additionally, the effects of these materials on soil acidity reclamation require detailed investigation. The current study aims to characterize the basalt rock dust originating from the Saurashtra region, India and to examine the impact of BRD addition on negative C emission in acidic soil. The mineralogical composition of BRD was analyzed through an X-ray diffractometer, and its behavior was continuously assessed in acid-aqueous solutions of different pH levels. An incubation experiment was performed for 128 days at 25°C, with eleven treatments with additions of lime, BRD, and organic matter (**OM**) to the soil in different doses and combinations. The treatments were Control (only soil), Lime (lime addition as per lime requirement), OM (addition of green manure and FYM), OM+BRD_{2.5} (addition of BRD @2.5 t/ha along with OM), OM+BRD₅, OM+BRD₁₀, OM+BRD₂₅, BRD_{2.5} (addition of BRD alone @2.5 t/ha), BRD₅, BRD₁₀, and BRD₂₅. The OM was used as a source of CO₂ emission through decomposition over time. The cumulative C emission (**C_t**) was measured periodically through the alkali trap method, and the effectivity of BRD applications on the reduction in the C_t was measured. A completely randomized design was used to analyze all the recorded data, with the analysis of variance (**ANOVA**) performed using R software.

The BRD was rich in feldspars and exhibited alkaline pH. It effectively neutralized the acidic aqueous solutions, especially those with lower initial pH. Treatments with only BRDs had C_t values similar to the control, while lime application significantly increased C_t ($p < 0.05$). The organic matter amended treatments, *i.e.*, OM, OM + BRD_{2.5}, OM + BRD₅, OM + BRD₁₀ and OM + BRD₂₅, showed higher C_t compared to the lime-amended treatment ($p < 0.05$). However, treatments with higher BRD doses (OM + BRD₅, OM + BRD₁₀ and OM + BRD₂₅) exhibited significant reductions ($p < 0.05$) in the C_t compared to that from the OM. The net negative C emission was plotted against the doses of BRD, and based on the break-even point of the graph, and statistical analysis of the slopes of the best-fitted trendline, the lowest dose of BRD corresponding to the highest negative slope was identified at 8 t ha⁻¹, which has a CDR potential of 356 Kg ha⁻¹, which, if extrapolated for potential use in pan-India acid soils, results in a CDR potential of 0.32 Gt.

The BRD has the potential to capture atmospheric carbon dioxide. The application of organics along with BRD facilitates the greater dissolution of silicate minerals and, in turn, the proper exploitation of their CDR potential. The application of BRD, instead of lime, offers a C-neutral reclamation technique for acidic soils.

82 Kinetics of microbial-consortium mediated decomposition of crop residues with different bio-compositions

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Globally, ~ 5 billion tonnes of crop residues (**CR**) are generated every year, which creates a major handling issue. The predominant method of managing these residues is field burning which releases mainly carbon dioxide (**CO₂**), contributing significantly towards greenhouse gas emissions. The eco-friendly and effective way of managing these CRs is *in-situ* composting in the crop field. The Indian Agricultural Research Institute, New Delhi, India developed “Pusa decomposer” (**PD**), a microbial consortium for quicker decomposition of different CR. The bio-composition of different CR can alter the decomposition of CR and, in turn, the C mineralization from inherent soil organic carbon (**SOC**) through “priming”.

This study investigates the influence of temperature, PD, and CR bio-composition on C mineralization and SOC priming. Additionally, it explores the relationship between quality attributes and decomposition rates of CR. Crop residues are analyzed for their biochemical and elemental composition. An incubation experiment was conducted with four different types of CR (rice, maize, cotton, and pigeon pea) in soil and soil-less nutrient solution at 25°C and 35°C, with and without PD addition. Carbon mineralization was measured using the alkali trap method at periodic intervals over 128 days. The C emission data was fit into a two-pool exponential model. Statistically, the data has been evaluated using a completely randomized design, using the R package.

Maize had the highest cellulose, while rice had the highest hemicellulose levels. Cotton registered the highest lignin and polyphenols, making it harder to decompose. At 25°C, maize showed the highest cumulative carbon mineralization (346 mg C g initial C⁻¹), whereas cotton registered the lowest (201 mg C g initial C⁻¹). The decomposability of the Rice CR increases significantly with an increase in the temperature, most probably owing to the abundance of lignin, holocellulose, and an assortment of volatile components contained therein. These components are most likely to be highly temperature-sensitive and result in a shift of the C-type made thereof, from recalcitrant to labile, consequential to the temperature increase. The addition of PD greatly enhances the decomposition of CRs ($P < 0.05$). The PD is more effective at 25°C than at 35°C, with the highest efficiency in decomposing pigeon pea crop residues, followed by rice, cotton, and maize. The temperature sensitivity (Q_{10}) of carbon mineralization varied among crop residues, with recalcitrant carbon being ~8.15% more temperature-sensitive than labile carbon. The PD addition increased Q_{10} values by ~15%, indicating enhanced temperature sensitivity of both labile and recalcitrant C. The addition of PD had an inverse effect on the priming of the inherent SOC, implying the protection of the latter.

The PD should be essentially included in the package of practices of different regenerative agriculture involving CR retention in fields. The microbially mediated decomposition of different CRs offers a win-win situation, *i.e.*, management of the huge CR load in agricultural lands and improvement in soil health.

90 Common emitters of greenhouse gases in Uganda

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Agricultural soils and grazing patterns are substantial contributors to greenhouse gas (GHG) emissions, primarily through nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) emissions with a global warming potential 298 times that of CO_2 . Key sources of emissions include nitrogen fertilization, poor soil management practices and enteric fermentation in livestock. Nitrous oxide is primarily emitted from soils due to synthetic and organic nitrogen applications exacerbated by poor management such as over fertilization while intensive tillage and land use changes can lead to significant soil carbon loss. Moreover, livestock contribute to methane emissions during digestion and through manure management particularly under anaerobic conditions. Microbial processes in soils are very important in the emission of these gases, influenced by factors such as vegetation type, soil moisture and nutrient availability. Several approaches have been used in measuring GHG emissions from agricultural soils and grazing patterns but the most suitable for this study in regard to agricultural soils were the chamber systems. These are on site methods where chambers are placed directly on the soil to measure gas fluxes. Chambers capture gases over a set period allowing for precise analysis. For grazing patterns the **GLEAM** (Global Livestock Environmental Assessment Model) were used which simulate emissions based on grazing intensity, Livestock type, feeding system and environmental factors. Atmospheric levels of GHG emissions varied; methane levels reached 11%, CO_2 levels 14% and Nitrous oxide levels were highest 75% in agricultural soils while GHG emissions in grazing systems accounted for 75% CH_4 , 14% CO_2 and 10% N_2O respectively. The rationale with land use systems is that croplands emit higher levels of Nitrous oxide due to fertilizer use. On the contrary grazing lands emit moderate levels of CO_2 and N_2O but high volumes of CH_4 through enteric fermentation per animal per year depending on the diet and management. Effective mitigation strategies include improving soil health through conservation tillage and cover cropping, enhancing pasture management by use of rotational grazing and optimizing nutrient management practices through precision agriculture and the use of alternate fertilizers. Integrating livestock and crop production can also foster nutrient cycling and reduce reliance on synthetic fertilizers. By adopting these strategies, it is possible to reduce GHG emissions from agricultural systems, contributing to enhanced sustainability and climate resilience.

116 Seasonal soil CO₂ efflux dynamics across land use systems in Ghana: implications for climate mitigation

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Carbon dioxide (CO₂) is a major greenhouse gas driving climate change. In Ghana, the Agriculture, Forestry, and Other Land Use (AFOLU) sector contributes to national CO₂ emissions while also offering opportunities for carbon sequestration. This study assessed seasonal variations in soil respiration rates (SRR) across four land-use types, including forest, fallow, maize, and rice fields within a semi-deciduous forest zone in Ghana. The aim was to provide baseline data and identify key soil factors that influence SRR to support mitigation strategies. Soil respiration rate was measured monthly over one year using a closed-chamber technique. Concurrently, soil physico-chemical properties such as organic matter (OM), pH, texture, and moisture (SM) were recorded. Stepwise multiple regression and correlation analyses were conducted to explore relationships between SRR and soil variables. SRR varied across seasons and land-use types, with higher emissions during the wet season. Fallow and cropland areas (maize and rice fields) recorded the highest CO₂ efflux, while closed forests showed the lowest emissions, likely due to minimal disturbance. Among the soil variables assessed, OM, pH, silt content, and SM showed stronger associations with SRR. The final regression model explained 58% of the variation in SRR.

This study shows that SRR vary by land-use type, with higher emissions observed in disturbed systems such as fallow and croplands, and lower emissions in undisturbed forest areas. These findings underscore the critical role of forest conservation and sustainable land management in mitigating greenhouse gas emissions and strengthening ecosystem resilience in tropical regions. To effectively reduce emissions from the AFOLU sector, policies should promote practices such as reforestation, agroforestry, and reduced soil disturbance that enhance soil carbon retention and support sustainable land-use transitions.

124 Application of dairy effluent on pecan plantations intensifies nitrous oxide and methane emissions

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Regenerative agriculture systems could reduce greenhouse gas emissions when efficiently integrated with silvopasture and dairy systems. However, little research has been conducted to understand the impact of dairy effluents on nitrous oxide (**N₂O**) and methane (**CH₄**) emissions, two of the most potent greenhouse gases (**GHGs**), from pecan-based silvopastoral systems, particularly under temperate climatic conditions. In this study, we aimed to estimate the effects of dairy effluent application on the emissions of GHGs from pecan-based silvopastoral systems in the Southern Pampas eco-region of Argentina under an Argiudoll soil type. Soil N₂O and CH₄ emissions were measured with static chambers in three treatments, including (1) proximity to a pecan tree with no dairy effluents (**PPT**); (2) proximity to a pecan tree with the application of dairy effluents (**PPT-E**); and (3) adjacent pastureland that was not shaded or fertilised as a control (**APL**). The study was conducted in a 10-year-old pecan orchard plantation between May and June 2023 and from January to February 2024. Dairy effluent was applied on May 9, 2023, and January 2, 2024, the PPT-E plots at a rate of 25.8 kg N ha⁻¹ in 2023 and 49.8 kg N ha⁻¹ in 2024 (5 L per tree with a N concentration of 42 and 81 mg L⁻¹). The mean accumulated emissions of CO₂ eq. ha⁻¹ were analysed under a mixed model with the lme4 package, accounting for the phenology by treatment interaction using the statistical program R studio v.4.4. In both seasons, the total emissions of N₂O and CH₄ under PPT-E were higher 58 kg CO₂ eq. ha⁻¹ than those under APL (37 kg CO₂ eq. ha⁻¹), although there was no significant difference. Due to the pecan tree phenology, the magnitude and composition of N₂O and CH₄ emissions varied with the season, with N₂O release predominating in winter and more CH₄ released in summer. Absorption or negative emission of N₂O during the summer, mainly in the soil near the pecan tree, was likely due to N₂O exhaustion in the soil matrix. There was a trend towards higher total accumulated emissions (N₂O + CH₄) during cold and wet periods, with pecan trees not demanding nutrients, and lower emissions during the warm and dry periods, with trees filling the nuts at high nutrient demand. Our results highlight season- and management-induced intensifying GHG emissions. Future research should focus on seasonal GHG flux across the year over a 2-3-year period to estimate the annual potential for offsetting CO₂ emissions in pecan orchards.

142 Greenhouse gas emissions from sheep urine application in New Zealand silvopastoral systems

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Agriculture is New Zealand's largest contributor of greenhouse gas (GHG) emissions, accounting for 53% of the country's net emissions in 2022. Grazing livestock contribute to nitrous oxide (N₂O) emissions, primarily from excreta deposition. While silvopastoral systems have been explored to enhance carbon dioxide (CO₂) removal by trees on pastoral farms, their potential to reduce N₂O emissions is less known.

To evaluate the effects of silvopastoral systems on soil GHG emissions (methane, CH₄ and N₂O), an experiment was established at the Ballantrae research farm (40.31572 S 175.83995 E, AgResearch Ltd, New Zealand) in September (spring) 2024. The experiment integrated the evaluation of tree density within a pasture system (open pasture with no trees, 121 and 420 poplar stems ha⁻¹, n=3) and sheep grazing, reflected as urine application (artificial urine, 4 L m⁻², 6 g N L⁻¹ ha⁻¹, n=9). A control treatment with no urine application was also included. Soil GHG emissions were measured using the static chamber technique (1x1.5 m² plots). Gas samples were taken prior to, and on days 1 and 3 after urine application, then twice a week for 4 weeks, and once a week until GHG emissions and soil available N reached background concentrations (70 days). Gas sampling was carried out at 0, 30 and 60 min. Gas measurements were complemented with soil available N (NO₃-N and NH₄-N) and moisture determinations, as well as other relevant site parameters (rainfall, temperature).

Averaged N₂O fluxes were lower in the control treatment (167 g N₂O-N ha⁻¹ d⁻¹) and increased significantly with urine application (8,575 g N₂O-N ha⁻¹ d⁻¹, across different land uses and tree densities). The peak of emissions in all treatments was observed 16-days after urine application, in response to soil NH₄-N nitrification following urine addition. This resulted in a 5-times increase in the cumulative N₂O emissions with urine (from 2.8 to 14.5 kg N₂O-N ha⁻¹). Emissions of N₂O increased with tree density (20 and 78% increase for medium (121 stems ha⁻¹) and high density (420 stems ha⁻¹) treatments, respectively, in comparison to the open pasture), independently of urine application. This was associated with greater soil moisture (2-3% v/v) and lower pasture yield (25-40% reduction, with lower N removal) in the tree treatments, in comparison to the open pasture, potentially resulting in greater N₂O emissions through the denitrification process.

Average CH₄ fluxes decreased with urine-N application (1332 and -55 g CH₄-C ha⁻¹ d⁻¹ for urine and control treatments across tree densities, respectively). The peak of emissions was observed six days after urine application, associated with greater cumulative rainfall over that period. Cumulative CH₄ emissions decreased with N addition, independently of the tree density, suggesting that CH₄ emissions were more closely related to soil N deficiency than to soil moisture.

Results suggest that the increases in N₂O emissions in high tree density areas, relative to open pasture, may offset some of the C sequestration benefits in silvopastoral systems. An overall carbon balance analysis could provide further insights into the benefits and trade-offs of silvopastoral systems in New Zealand.

145 Methane emission of dairy cows grazing versus indoor feeding with silage

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Recent studies show that methane emission may be lower during grazing compared to feeding a ration or silage indoors. Yet, reasons for this difference remain unclear. This study aimed to quantify enteric methane emission of Danish Holstein dairy cows grazing perennial ryegrass and white clover pasture compared to dairy cows fed grass-clover silage indoors, while exploring the underlying mechanisms (not presented) for the potential methane reduction. Methane emission was measured with the GreenFeed system (C-Lock Inc., USA) indoors (2 units) and on pasture (2 units). In a continuous design, 45 intact lactating dairy cows were allocated to one of 3 treatments (15 cows/treatment): 1) indoor feeding (day and night) with a spring growth (first cut) grass-clover silage, 2) fulltime grazing (day and night), and 3) parttime grazing (day, ca. 8 h) with indoor feeding of silage (night). All cows were allowed up to 3.0 kg/d of fresh concentrate in GreenFeed and received 2.7 kg/d of fresh concentrate in the milking parlor. The experiment started with spring growth and included 2 weeks of adaptation and 9 experimental weeks (May 6th to July 7th). Cows grazed pasture in a rotational system over paddocks and using strip grazing within paddock. Pasture measurements were taken with Grasshopper (True North Technologies, Ireland) pre- and post-grazing a paddock. The pre-grazing herbage mass was ca. 1240 kg DM/ha, and the pre- and post-grazing sward heights were ca. 98 and 66 mm. The number of weekly visits per cow to the GreenFeed units were on average 26, 15, and 23 visits/week for the indoor, fulltime grazing and parttime grazing treatments, respectively. The preliminary results indicate that energy-corrected milk (**ECM**) yield, milk fat content, and methane emission (g/d and g/kg ECM), were lower for fulltime grazing cows compared to the indoor treatment. Parttime grazing cows, had the highest ECM yield, and a milk fat content and methane emission (g/d and g/kg ECM) intermediate to the indoor and fulltime grazing treatments. This work is ongoing, therefore more detailed results on methane emission, milk yield and composition, and calculated dry matter intake will be provided at the conference. The preliminary conclusion is that cows grazing, especially fulltime, have a lower methane emission compared to cows fed with spring growth grass-clover silage indoors.

148 Nitrous oxide emissions under conservation agriculture and biochar amendments in a Ferralsol, northern Uganda

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In sub-Saharan Africa (**SSA**), N₂O emissions are mainly associated with forest clearing, livestock manure, and crop production (Boateng et al., 2019). Although acidic soils tend to have high N₂O emissions (Wang et al., 2018), the limited N inputs in smallholder farming systems in SSA reduces soil N availability, consequently leading to relatively low N₂O emissions. The recent 2024 Nairobi Declaration, targeting increased fertilizer use in Africa (Africa Union, 2024), might change future trajectories of fertilizer consumption in SSA, potentially increasing soil N availability and N₂O emissions over time. Conservation agriculture (**CA**) and biochar amendments have been proposed to enhance crop yield and soil fertility, and to reduce N₂O emissions (Zhang et al., 2021). However, little is known about how they affect baseline N₂O emissions in unfertilized systems.

Effect of CA and biochar amendments on N₂O emissions on an unfertilized Ferralsol in northern Uganda was investigated. Specifically, we compared crop rotation (pigeon pea – maize) with maize monocropping under conventional tillage. In addition, we compared pigeon pea – maize rotation under three practices, i.e. conventional tillage, CA (reduced tillage), and CA in combination with biochar (CA+biochar). We hypothesised that rotation with pigeon pea increases N₂O emission compared to maize monocropping, while CA+Biochar reduces N₂O emissions, both compared to CA and to conventionally tilled soil. To mimic subsistence farming systems in Uganda, no inorganic fertilizer was applied.

Custom-made PVC chambers manufactured from 16 cm diameter, grey opaque sewage pipes with a self-sealing rubber septum were used to sample N₂O emissions. For each flux measurement, four gas samples were drawn from the chamber headspace 1, 15, 30 and 60-minutes after deployment using a 20 mL polypropylene syringe equipped with a three-way valve. Gas sampling was done biweekly, resulting in 17 sampling campaigns between May 2023 and January 2024. The samples were analyzed using gas chromatography connected to an auto-sampler, where N₂O was quantified by an electron capture detector.

N₂O fluxes were small, ranging from 1.02 – 51.19 µg N m² h⁻¹ over the entire period. Short-lived emission peaks were observed following pigeon pea harvest in the crop rotation, which were absent in maize monocropping. Overall, across all seasons, cumulative growing-season (279 days) N₂O emissions ranged from 0.44 – 1.11 kg N ha⁻¹. Biochar amendments in CA systems did not affect daily and cumulative emissions. In the first season, yield-scaled N₂O emissions and N yield scaled N₂O emissions were significantly smaller in CA systems with biochar compared to conventional tillage, suggesting that CA and biochar was effective in minimizing emissions without penalizing pigeon pea productivity.

166 White clover's role in reducing nitrous oxide emissions in grazing swards in dairy cow systems

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Inorganic nitrogen (**N**) fertiliser is a major contributor to greenhouse gas (**GHG**) emissions through nitrous oxide (**N₂O**) losses. The Irish Government has set a target to reduce GHG emissions by 51% by 2030 compared to 2018. The Irish agricultural industry accounts for 37% of total GHG emissions, of which 10.6% comes from N fertilisers. The level of N₂O emitted is critical as N₂O is 298 times more potent than CO₂ and persists in the atmosphere for a lengthy time (> 100 years). Grazed grass is the cheapest feed available for dairy cows in temperate regions thus to maximise profits, dairy farmers must utilise this high quality feed where possible. To reduce cost inputs and environmental impacts of inorganic N use, there is renewed interest in the incorporation of legumes, white clover (**WC**) in particular in grazing systems. The objective of this study was to reduce chemical N fertiliser input to pasture based milk production systems by incorporating WC into perennial-ryegrass swards to maintain herbage and milk production.

The experiment was undertaken at Moorepark, Cork, Ireland from February to November in 2023 and 2024. Three grazing treatments were used for this study; grass-only swards receiving 200 kg N/ha (**Gr200**) and grass-WC swards receiving 100 (**CI100**) or 150 kg N/ha (**CI150**) annually. Twenty cows were assigned to each treatment and swards were rotationally grazed at a stocking rate of 2.4 cows/ha. Pre-grazing herbage mass in each paddock was determined weekly. Sward WC content was measured before grazing in each paddock each rotation. Milk yield was recorded daily and milk composition weekly by taking milk samples from a consecutive evening and morning milking. Nitrous oxide annual fluxes were estimated based on previous measurements and conditions. Data from 60 cows over two years (120 variables) were available for analysis. Grazing characteristics were analysed using Proc MIXED linear model in SAS (SAS 9.4). Terms included in the model were paddock (subject), rotation (repeated), WC treatment and fertiliser rate treatment. Milk data were also analysed using Proc MIXED in SAS (SAS 9.4). Terms included in the model were sward type treatment, fertiliser rate treatment and parity.

Cows grazing the grass-WC treatments had greater production ($P<0.05$) in terms of milk yield (+230 kg) and milk solids yield (+32 kg) compared with Gr200 treatment. The grass-WC swards produced 5,878 kg milk and 536 kg milk solids (kg fat + protein) per cow. There was no treatment effect on herbage production, 13.1, 12.9 and 12.7 tonnes DM/ha for Gr200, CI150 and CI100 systems, respectively. Annual WC content was 20%. It is estimated that the N₂O annual flux for Gr200 is twice as high as the CI100 (approx. 4 kg N₂O-N/ha vs 2 kg N₂O-N/ha annually, respectively) due to their exponential relationship and timing of chemical N applications with CI150 treatment intermediate.

Nitrous oxide emissions through grazing systems could be reduced by half by substituting chemical N fertiliser inputs with BNF from WC while maintaining herbage production and additionally increasing milk production.

170 A life cycle assessment and net protein contribution of cow-calf operations in Florida

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Florida's cow-calf operations play a crucial role in U.S. beef production, yet their reliance on extensive grazing systems presents significant challenges in achieving economic viability and environmental sustainability. This study assessed the greenhouse gas (GHG) emissions and protein contributions of 16 cow-calf farms across Florida using a life cycle assessment (LCA) and net protein contribution (NPC) framework. Data were collected through comprehensive farm surveys, capturing variability in management practices and herd characteristics across diverse regions. Emissions of methane (CH₄), nitrous oxide (N₂O), and carbon dioxide (CO₂) were analyzed, considering both on-farm and off-farm activities. Enteric fermentation emerged as the dominant source of CH₄ emissions, accounting for 97.7% of total CH₄ output, reflecting the reliance on high-fiber diets typical of extensive systems. Nitrous oxide emissions were primarily derived from manure deposited on pastures (52.9%), with fertilizer use contributing 16.8% directly and 6.9% indirectly. The CO₂ emissions were largely driven by the production and transport of fertilizers (66.1%), while fuel and electricity contributed minimally. When all gases were converted to CO₂ equivalents (CO₂e), enteric methane accounted for 70% of total GHG emissions, underscoring its significance. Nitrous oxide emissions, largely linked to manure and fertilizer applications, constituted 18%, with CO₂ from off-farm activities contributing to the remainder. Emissions intensity ranged from 17.5 to 28.4 kg CO₂e per kg of weaned calf, with more efficient farms exhibiting lower values. Productivity per hectare, measured as weight gain or weaned calves per hectare, was inversely correlated with emission intensity, highlighting the potential for sustainable intensification. Cow body weight showed a positive correlation with GHG emissions intensity, as mature cows contributed approximately 80% of total emissions due to their significant nutrient and energy demands, reflecting the trade-offs inherent in extensive systems. The NPC analysis revealed notable variability in human-edible protein conversion efficiency (HePCE), which ranged from 2.1 to 98.2 across farms, influenced by management practices and feed supplementation. Protein quality, evaluated using the Digestible Indispensable Amino Acid Score (DIAAS), varied from 5.0 to 56.6 for feedstuffs, compared to 112 for beef, emphasizing the superior nutritional value of the final product. NPC values ranged from 4.4 to 434.3, with all farms achieving positive values (NPC > 1), demonstrating that these systems contribute to human protein requirements without competing for edible feed resources. Land use efficiency, expressed as hectares per ton of human-edible protein produced, ranged from 9.1 to 76.2 ha. Farms employing strategic supplementation exhibited better land use efficiency, even when their NPC values were lower. These findings underscore the potential for targeted management strategies to enhance the sustainability of cow-calf systems. Farms that optimize productivity per hectare, manage cow body weight, and incorporate dietary improvements can reduce GHG emissions while maintaining or enhancing protein efficiency and land use. This research provides actionable insights to align beef production with global food security and climate mitigation goals, balancing the need for high-quality protein with reducing the environmental footprint of livestock systems.

181 Effect of pasture quality and availability on ruminants methane emissions under Nordic conditions

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In Norway, agriculture accounts for about 8% of total greenhouse gas emissions, of which around 50% is assumed to be enteric methane. Red meat production has high GHG emissions (/kg product, meat/milk) where dairy cows and sheep account for more than 80% of these emissions in total. Among several strategies to reduce methane emissions from ruminants, improved feed quality is very important. Grazing is central to Norwegian livestock farming. Sheep are grazed after lambing and remain outside either on cultivated pasture or rangelands, until slaughter and mating in the autumn (October/November). It is estimated that Norwegian sheep themselves harvest a good 50% of their annual consumption on pasture. Norwegian dairy cows are required to have a minimum of six weeks of grazing or exercise outdoors during the year.

Three grazing trials with sheep or dairy cattle were carried out under Nordic conditions included measurements of CH₄ related to pasture quality, proportion of forage intake (fresh vs. conserved forage in the total ration), and time of grazing (day or night). The sheep trial included 16 ewes grazed for two periods of 10 days (July and August) with an expected difference in pasture quality between periods. The study used an in vitro gas technique approach and modelling to estimate CH₄ production and fermentation. The results suggest that pasture quality is an important factor to consider when implementing grazing strategies. Both dairy cow experiments used GreenFeed units indoors and outdoors to measure CH₄.

The first dairy cow experiment investigated the effects of turning parlour-milked cows to pasture during either daytime or night-time on animal performance and CH₄ emissions. Thirty Nordic Red cows were divided into two groups, one grazing during the day and one grazing during the night. Cows were milked in the stall and kept indoor when not on pasture. Time of grazing had no effect on total dry matter intake, milk production or CH₄ emissions. However, CH₄ emissions dropped by 28% when cows were on pasture (293 g CH₄/day) compared to being indoors (413 g CH₄/day).

The second dairy cow experiment investigated the effects of allowing cows milked in an AMS, free roaming between the barn and either a production pasture or an exercise pasture. Production pasture was aimed to provide 50% of the animals' daily dry matter intake, while no feed intake was expected from the exercise pasture. In a change-over design, 32 Norwegian Red cows had two weeks adaptation followed by two weeks of recording within each treatment. There were no differences in daily dry matter intake or energy-corrected milk production between treatments. Cows given access to the production pasture produced 322 g CH₄/day while cows with restricted pasture access produced 387 g CH₄/day.

The results highlight the value of grazing in the feed ration of sheep and dairy cows. Fresh grass has a quality superior to conserved forage. We conclude that grazing is an important measure to reduce greenhouse gas emissions from ruminants under Nordic conditions and detailed results will be presented at the conference.

183 Pathways to mitigate greenhouse gas emissions in temperate grazing systems

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Carbon accounting in the agricultural sector, particularly within the red meat industry, presents complex challenges due to evolving standards, schemes and policies, differences in the scope of emissions considered, and the variable impacts of climate conditions and management on grazing systems. Greenhouse gas (**GHG**) emissions accounting frameworks often inadequately represent the dynamic interactions between farm components and environmental conditions, which impacts their accuracy and effectiveness of mitigation strategies. This study aimed to develop an integrated framework to quantify net GHG emissions (**NE**) and emissions intensity (**EI**) and to identify evidence-based mitigation strategies tailored to site-specific farm dynamics. We established a system boundary to analyse these variations, combining a partial pre-farm life cycle assessment (**LCA**) with whole-farm GHG accounting, which included emissions from manufacturing farm inputs and emissions associated with on-farm activities up to the farm gate. The framework was applied to a single grazing beef and sheep farm located in a winter-dominant rainfall agroecological region in northeastern Victoria (**NEV**), Australia. Historical production and management records (2013–2023) were collected through interviews with farm managers. Key farm management data included average annual stocking rates (10–12 DSE/ha), grazing management, pasture composition, fertiliser use, supplementary feeding, and sales inventory. Farm-level emissions were estimated using the Greenhouse Gas Accounting Framework (**GAF**) tools consistent with the Australian National Greenhouse Gas Inventory methodology. For sheep systems, emissions were allocated between wool and meat using protein mass allocation (**PMA**). Results were expressed as tonnes of carbon dioxide equivalents (**CO₂e**) at the farm scale and EI as kilograms of CO₂e per kilogram of product sold (kg of liveweight for beef, sheep meat, and kg of greasy wool). The Sustainable Grazing Systems model (SGS Version 5.6.3) used climate and measured soil data to quantify pasture, animal production, and soil carbon dynamics. Carbon stock changes from tree plantings were estimated using FullCAM, which included above and below-ground tree biomass. Mitigation measures were modelled using two trajectories: 'lower adoption rate' (**LA**), aligned with current practices, and 'higher adoption rate' (**HA**), representing a more ambitious adoption scenario. Selected mitigation strategies were based on regional scientific evidence, alignment with existing farming systems, and their potential for adoption under current management. This approach integrated site-specific environmental and management conditions, evaluating the co-benefits and trade-offs of each strategy to capture their interactions and effects. Both approaches improved livestock production and reduced historical NE and EI. For NEV, EI ranged from 10.2–16 kg CO₂e/kg LW for beef (± 1.97 , CV 15%), 7–12 kg CO₂e/kg LW for sheep meat (± 1.54 , CV 18%), and 22–24 kg CO₂e/kg greasy wool (± 6.05 , CV 19%). The HA achieved more significant NE reductions, while the LA, aligned with current farm practices, resulted in lower average NE reductions: 10% for feed additives, 3% for early programming (**EP**), and 8% through the establishment of anti-methanogenic pastures (**AP**). In HA, feed additives reduced NE by 25%, EP by 16%, and AP by 17%. The tree plantings within the farm system decreased NE by 8.7% (LA) and 43.5% (HA).

194 Partial carbon footprint of Uruguayan dairy farming

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In the last decade, livestock's responsibility in terms of its contribution to greenhouse gas (GHG) emissions has been placed on the global agenda. Worldwide, GHG emissions from the agricultural sector account for 18.4% of total emissions. Uruguay presents a distinct profile, where the sectoral proportions are reversed compared to international references: the agricultural sector accounted for 73% of the National Greenhouse Gas Inventory in 2019. This poses a challenge for beef and dairy production, which must respond to national and international public opinion with clear and transparent evidence. On the other hand, showcasing the environmental performance of production systems could provide an opportunity by contributing to the supply of differentiated goods in response to increasingly sophisticated consumer preferences for the process attributes behind food production.

In 2021, a technical group of experts was established, bringing together representatives from the Ministry of Livestock, Agriculture and Fisheries, the Ministry of Environment, the National Institute of Milk, the National Institute of Meat, the National Institute of Agricultural Research, and the University of the Republic. This inter-institutional group, known as the Environmental Footprint of Livestock Group, was tasked with calculating and analyzing the environmental footprint of beef and dairy livestock in Uruguay.

This study aims to estimate the partial carbon footprint of the Uruguayan dairy production phase, using a partial life cycle approach (cradle to farm-gate) that considers the processes and inputs involved in milk production. Data from the 2019 Dairy Survey was used. The survey is based on a sample of 294 producers representative of Uruguay's commercial dairy sector. It includes information on dairy herds, production, reproduction and rearing, land use, forage and crop production technologies, feed and supplement purchases, as well as milking and effluent management infrastructure.

The average carbon footprint value of the dairy production process was 0.989 kilograms of CO₂ equivalent per kilogram of milk corrected for fat and protein contents (kg CO₂e/kg FPCM). The analysis of farm clusters by intensification level showed a carbon footprint ranging from a minimum of 0.883 to a maximum of 1.387 kg CO₂e/kg FPCM. A negative correlation was observed between milk productivity and the carbon footprint: the more productive a farm, the smaller its carbon footprint. Emissions from enteric fermentation accounted for half of the carbon footprint, followed by nitrous oxide emissions (26%) from feces and urine deposition, and methane emissions from effluent management (9.7%). GHG emissions related to herd management accounted for 86% of the carbon footprint of the overall process. At the international level, Uruguayan production is positioned relatively favorably. According to recent documented standardized systematizations, the global average from results published for 19 countries was 1.37 kg CO₂e/kg FPCM, ranging from 0.74 to 3.29 kg CO₂e/kg FPCM.

This study contributes to the generation of national information on a topic that is relevant from both environmental and commercial perspectives. The accumulation of knowledge in this regard is necessary for fostering social dialogue on the debate surrounding agri-food systems and their interactions with natural resources and the environment.

218 GHG emissions from cattle urine patches in two pasture-based dairy systems with contrasting intensification strategies

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The intensification of dairy production is crucial for meeting the growing global demand for dairy products. However, this process raises environmental concerns, particularly regarding greenhouse gas (GHG) emissions adding to global warming. Livestock systems contribute significantly to agricultural emissions, with pasture soils being a major source of nitrous oxide (N₂O) due to nitrogen deposition from animal urine. This study assessed soil emissions of N₂O, CH₄, and CO₂ in two contrasting pasture-based dairy systems designed as sustainable intensification strategies: (1) **OS** (Operational Simplicity), with a stocking rate (**SR**) of 1.8 LU/ha and minimal external inputs; and (2) **HP** (High Productivity), with an SR of 3 LU/ha and greater reliance on external inputs. The trial was conducted during winter 2023 at the CRS research station (Faculty of Agronomy, UDELAR, Canelones, Uruguay). Emissions were measured over eight weeks using closed static chambers, with gas-sampling conducted three times per week, on average. A total of twelve chambers were used (three per treatment per system). Treatments included: (a) fresh urine, applied at 1 L/m² (equivalent to 57 kg N/ha, collected from cows in each system), and (b) control, with water applied at the same volume to match moisture conditions. Urine was collected during morning milking from cows in each respective system – HP urine from HP cows, and OS urine from OS cows – immediately prior to application. The applied dose was based on values reported in previous studies. Statistical analysis was performed using *gasfluxes* package in R. Preliminary results showed no significant differences in GHG emissions between systems ($P > 0.05$). In the urine treatment, cumulative winter emissions averaged 0.6 kg N₂O-N/ha, -3.4×10^{-5} kg CH₄/ha, and 0.12 kg CO₂/ha. Both systems exhibited similar soil GHG emission profiles under winter conditions, suggesting that sustainable intensification can be pursued without increasing soil-derived GHG emissions. Ongoing work will assess seasonal variability and refine the accuracy of carbon footprint estimates in pasture-based dairy systems.

220 Grazing management increases milk production and mitigates methane in Colombia

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Height-enhanced grazing (Pama in Colombia or Rotatinuo in Brazil) is based on improved pasture structure by improving the ingestive behavior of grazing animals. This strategy promotes faster and higher forage intake, increasing production and decreasing enteric methane emissions. The purpose was to test two grazing management strategies: traditional rotational (Tr): with target forage canopy heights before grazing of 40 cm (high grazing intensity (stocking rate) with low grazing frequency (rest)), and Pama with a forage canopy height before grazing of 20 cm (medium intensity, with high frequency), in kikuyu (*Cenchrus clandestinus*) pastures with Holstein cows.

Holstein cows were used for milk production in the municipality of San Pedro, Antioquia province, Colombia, on the La Florida farm, located at 2098 meters above sea level, with an average temperature of 16.6°C and an average rainfall of 2500 mm/year, belonging to an ecological zone of Humid Forest - Low Montane (bh - MB). The experimental design was a Cross Over, with two periods each of 12 days (seven of accustoming and five of measurement), with six experimental units (cows) per treatment. Twelve multiparous Holstein cows were used with an average live weight (LW) of 483 kg, which were 138 ± 12 days in lactation and with an average production of 22.5 L/day. The experimental area was 1.5 ha divided into eight equal paddocks (1875 m²).

The pasture was fertilized with 275 kg of N₂O and 125 kg of P₂O₅/ha/year. Grazing management was based on grazing a plot of 1875 m²/day of occupation. Pasture management was in accordance with the pasture heights proposed in the evaluation (Pama, 20 vs Tr=40 cm). Forage supply in the pastures was determined using the double sampling technique and canopy height was measured using Sward Stick with 60 measurements per pasture. Grazing intake per animal was measured using chromium oxide as an external marker and indigestible NDF as an internal marker. Feed supplementation was 4 kg/cow/day with a commercial concentrate. Milk production per animal/day and its compositional quality were measured. Daily CH₄ emissions were determined using the sulfur hexafluoride tracer technique. The SF₆ permeation tubes used had average permeability rates of 5.6148 mg/day and were deployed in the reticulum of the animals (oral dosing), 20 days before the start of the measurements.

Forage supply (kg DM/ha) was the same ($P>0.05$): 2779 Pama vs 2909 Tr. Forage intake was similar in Pama (16.44) vs Tr (16.38 kg DM/cow/day). The nutritional quality of the forage from the kikuyu pasture, in both treatments was similar ($P>0.05$) for NDF, ADF, CP, OM and IVD. Milk production was higher ($P<0.05$) in Pama (22.05) vs Tr (18.52 kg/cow/day). The compositional quality of milk was different ($P<0.05$) for protein (2.82 Pama vs 2.73% Tr), but there were no differences in the other quality parameters. Enteric methane emissions were lower ($P<0.05$) in the Pama treatment (295.2) vs Tr (409.3 gr/cow/day), which implies a CH₄ emissions efficiency of 13.39 gr/L of milk in Pama and 22.10.

237 GHG flux measurements in grasslands for assessing the sustainability of boreal milk production

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Livestock management depends on grasslands for forage production for livestock. For a complete environmental assessment of milk production, all CO₂ equivalent costs including those associated with grassland management need to be accounted for. While methane emissions from cattle enteric fermentation dominate the livestock husbandry sector, CO₂ and N₂O emissions are dominant from grassland primary production perspectives. Typically, under boreal conditions (e.g., in Finland) legume grass species are cultivated on mineral soils, while nonlegume grasses are cultivated on drained organic soils over a short rotation of 3-4 years. Routine life cycle assessments of milk production assume that grasslands are carbon neutral as the emissions associated with grass cultivation are thought to equal the amount of CO₂ sequestered during the rotation cycle. However, based on several years of experience with year-round, continuous GHG flux measurements made with the eddy covariance method, it is obvious that GHG fluxes are a function of prevailing climatic conditions, grass species and soil types and grassland management practices. This presentation will showcase the multi-year GHG flux measurements from grasslands cultivated on mineral and drained organic soils in Finland as relevant to the sustainability of milk and beef production in Finland. Based on our initial analysis of the data from the year 2022, the legume grassland on a mineral soil site was a smaller source of CO₂ compared to the two drained peat soils that emitted high amounts of all three GHGs into the atmosphere. These drained peat soils also failed to produce high amount of grass biomass. Thus they were found to be not only environmentally unfriendly but also economically non-viable. Such observations, although based on a single year measurements, lead us to ponder whether grassland management on drained organic soils is sustainable from a climate perspective. Forage production on such soil types leads to high cost to the environment per liter of milk or kg of beef sold in the markets. For future studies, long-term studies across a broader range of boreal grassland sites, soil types, and management practices are essential to better capture the inter-annual variability and establish more robust, region-wide recommendations. Such research would better inform farm and landscape level grassland modelling, policy and farm-level decisions, supporting the dual goals of productive agriculture and climate change mitigation in boreal regions.

239 Effects of irrigation and fertilizer management on greenhouse gas emissions in rice fields

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Global warming potential (**GWP**) and carbon equivalent emissions (**CEE**) are influenced by greenhouse gas (**GHG**) emissions from rice cultivation, which include carbon dioxide (**CO₂**), methane (**CH₄**), and nitrous oxide (**N₂O**). This study evaluated the effects of irrigation regimes and fertilizer management practices on GHG emissions, GWP, and CEE in a rice field. The experiment was laid out in a split-plot design with irrigation regimes (Alternate Wetting and Drying (**AWD**) and Continuous Flooding (**CF**)) as main plots and fertilizer treatments as subplots. Fertilizer treatments consisted of two forms (granulated NPK + granulated urea (F1) and granulated NPK + urea super granule (F2)) and three placement methods (side or band placement (P1), broadcasting (P2), and deep placement (P3)). GHG fluxes were measured using the static chamber technique, while GWP and CEE were computed based on emission rates and grain yield. Data were analyzed using analysis of variance (**ANOVA**) to determine significant treatment effects, with Tukey's post-hoc test applied at a 5% significance level. The findings demonstrated that AWD decreased greenhouse gas intensity (**GHGI**) (0.13 vs. 0.32 kg CO₂ eq kg⁻¹ grain production), CEE (324 vs. 510 mg CO₂ eq m⁻² day⁻¹), GWP (1,187 vs. 1,871 kg CO₂ eq ha⁻¹), and CH₄ emissions (1.82 mg m⁻² day⁻¹) in comparison to CF (11.40 mg m⁻² day⁻¹). Forms and placement methods were shown to have trade-offs in fertilizer management approaches. Deep placement (P3) with granulated NPK + urea super granule (F2P3) reduced GWP (1,224 kg CO₂ eq ha⁻¹), CEE (334 mg CO₂ eq m⁻² day⁻¹), and GHGI (0.17 kg CO₂ eq kg⁻¹ grain yield), while deep placement (P3) with granulated NPK + granulated urea (F1P3) produced the highest CH₄ and N₂O emissions (13.40 and 7.32 mg m⁻² day⁻¹, respectively) and GWP (2,516 kg CO₂ eq ha⁻¹). Compared to livestock systems, where enteric fermentation is a dominant source of CH₄ emissions, or grasslands that act as both carbon sinks and emission sources, rice cultivation presents unique mitigation challenges and opportunities. The AWD method significantly reduces CH₄ emissions compared to continuous flooding, suggesting that its adoption could contribute to broader agricultural GHG mitigation strategies. Results from this study show that AWD irrigation combined with optimized fertilizer placement, particularly deep placement with granulated NPK and granulated urea, effectively reduces GHG emissions while maintaining rice productivity. This offers a practical pathway to climate-smart agriculture.

257 Nitrous oxide emissions from urine patches of beef cattle grazing poly crop mix swath

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Nitrous oxide (**N₂O**), a potent greenhouse gas (**GHG**), can be emitted from grazing systems during two distinct stages of production: forage growth via soil nitrogen (**N**) transformations, and animal grazing phase through excreta deposition and transformation in the soil. In order to achieve net-zero GHG emissions by 2050, N₂O emission reductions are needed. As such, evaluation of Beneficial Management Practices (**BMP**) with potential to mitigate N₂O emissions from the Canadian agricultural sector has gained interest. Poly-cropping (i.e., growing mixed annual forage species) has been practiced as a BMP to promote high resource use efficiency and influence edaphic-climatic conditions, which are major controllers for soil N₂O emissions. However, limited information is available on the impacts of swath grazing of poly-crop blends on urine N concentration and urine-derived N₂O emissions, particularly in semiarid climates. This study was conducted around Swift Current, Saskatchewan, where some of the driest parts of the Canadian Prairies exist. Three treatments, in a completely randomized block design ($n = 3$), were evaluated: i) control (forage oats monocrop), ii) simple blend: four forage specie mix (forage oat, forage pea, forage brassica, and hairy vetch), and iii) complex blend: eight forage specie mix (simple blend plus forage barley, red proso millet, teff grass, and chicory). Each experimental unit consisted of 1.66 ha paddocks, seeded on June 5, 2023, and swathed in August 2023 once the oat reached the hard dough stage. A total of 36 dry pregnant beef cows ($n = 4$) were used for the grazing component (initial weight = 593 ± 52 kg) between October 11 and November 17, 2023. Urine samples were collected from the grazing cows on October-25, 2023 and analyzed for total-N, urea-N, and ammonium (**NH₃**). Part of the collected urine (10L m⁻²) was applied back to a microplot immediately to simulate a typical cow urination event. The N₂O emission measurement commenced a day after urine application using the static chamber method and continued for one year. Swath yield varied among treatments, with the complex blend yielding the highest (3898 kg DM ha⁻¹, equivalent to 71.1 kg N ha⁻¹) followed by simple and monocrop (3268 and 2630 kg DM ha⁻¹, respectively). Urine total-N (average 12.4 g/L) and NH₃-N (average 1.4%) were not affected by treatments. However, urea-N was marginally affected by treatment, with higher values for poly crop treatments than monocrop (75% vs. 42% of total-N). Annual cumulative N₂O emissions since urine application, estimated by summing the background flux, 10 urinations/animal/day, 38 grazing days, at 4 animals/paddock, ranged between 610 and 790 g N₂O-N ha⁻¹ but was not affected by treatments. This translated into an emission factor for urine ranging from 0.69 to 0.74%, higher than values previously reported in the literature for semiarid climates. The above-normal precipitation that occurred in May-2024 and the proximity to when the land use was changed from perennial forage into annual forage for this trial may have contributed to the observed higher emission factor. These results are preliminary as the study will continue until 2026.

270 Rotation diversity and nitrogen source interactions on N₂O emissions from livestock feed production

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Agricultural lands in eastern Canada, with its cool humid climate, have recently seen a trend where perennial forages (primarily grass swards) are replaced with annual crops; in particular a rotation including corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.), for livestock feed. Perennial forages in rotation with annual crops, however, can improve agricultural resilience by increasing soil organic carbon (C). The application of livestock manure, which is common for dairy production systems in the region, can also increase soil organic C. However, interactions between diverse rotations and nitrogen (N) source (i.e. manure versus mineral N) on production and soil greenhouse gas (GHG) emissions are not well understood.

To investigate how these interactions affect growing season soil nitrous oxide (N₂O) emissions and surface soil (0-0.10 m depth) organic C concentrations, we compared five years of either a corn-soybean-corn rotation or a mixed perennial grass sward, followed by corn-soybean in all treatments for the last two years of a 7-year rotation at a site near Quebec City, Canada. All crops received N as either a mineral fertilizer (MIN) or a liquid dairy manure (LDM); N-fixing alfalfa (*Medicago sativa* L.) was also included to the perennial grass sward as an additional treatment. The experimental design was a split-plot with four replicates. The first three years of the 7-year rotation were also investigated at two other sites in eastern Canada (Truro, Nova Scotia; and Ottawa, Ontario). The full 7-year rotation was only measured at the Quebec City site.

When summed across sites over the first three years, cumulative growing season N₂O emissions were greater for LDM than MIN in the annual crops, but not in the perennial forages. Over the first three years, the MIN treatment generated greater yields than LDM in both annual and perennial crops, but had lower yield-scaled emissions (i.e. emissions per kg of product) in annual crops only. Over the entire 7-year rotation, N₂O emissions were consistently lower in the perennial crops compared to annual crops. Termination of the perennial crops at the end of year 5 (autumn 2020) however, led to a large peak of N₂O the following spring, the highest emission event in any of the treatments. However, cumulative growing season N₂O emissions for the entire 7-year rotation were still lower from the rotations that included perennial crops compared to rotations with only annual crops. Winter measurements on a sub-set of the plots showed low emissions during that period and no noticeable difference between treatments. Over the first three years at all three sites, the “active” fractions of soil organic C were more responsive than bulk soil organic C to the different treatments with initial findings suggesting more C storage in the soils under perennial crops and receiving LDM, compared to annual crops and MIN.

These findings suggest that including perennial forage crops in rotation with annual forage crops can reduce GHG emissions and improve soil C functioning while maintaining sufficient production of high-quality dairy feed.

277 Assessment of enteric methane emissions and animal performance of Blanco Orejinegro cattle in Antioquia department

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Colombia has been ranked in first place in bovine diversity in Latin America due to its seven creole cattle breeds, including the Blanco-Orejinegro (**BON**). BON is a dual-purpose breed raised in grazing-based system in the Antioquian Inter-Andean Valley on tropical grasslands. It is recognized for its adaptability, including rusticity, high reproductive efficiency, and tolerance to heat and humidity. However, its potential for enteric methane emission has not been previously assessed. The aim of this descriptive study was to evaluate animal performance, including daily weight gain (**DWG**, kg.d⁻¹), total dry matter intake (**TDMI**), enteric methane emission (**eCH₄**, g.d⁻¹), CH₄ yield (eCH₄.DWG⁻¹, g.kg⁻¹), in eight BON steers (348.5±90.6 kg, and 22.6±6.4 months) grazing heterogeneous pastures composed of *Paspalum sp.* and *Urochloa decumbens*, supplemented with an energetic-proteic concentrate (Forage:concentrate, 80:20). The steers had a management adaptation period of 21 days, followed by three experimental periods of 21 days. These periods were comprised by 14 days of titanium dioxide (**TiO₂**) dosing (10 g.d⁻¹) as an external marker to determine DMI, followed by 5 days of faecal sample collection, and enteric methane emissions that were measured six times daily per animal at 02:00, 06:00, 10:00, 14:00, 18:00 and 22:00, using the laser methane detector (Crowcon Mini-G). Feed quality was estimate by NIRS. Feed digestibility was determined using the *In vitro* dry matter digestibility (**IVDMD**) method, and the indigestible neutral detergent fibre (**iNDF**) content of forage, concentrate and faecal samples was determined through *in vitro* fermentation (288h). Statistical analysis was performed using RStudio 4.3.3 software. Pearson correlation coefficients were calculated to identify potential trends between variables. Results showed an IVDMD of 595.6 g.kg⁻¹ for forage and 917.5 g.kg⁻¹ for concentrate. The iNDF determined for forage, concentrate and faecal samples were 16.47, 2.07 and 51.42 g.100g⁻¹ of NDF, respectively. Mean Intake were of 9.1±2.6 kg.d⁻¹ for TDMI, 7.3±2.6 kg.d⁻¹ for forage and 1.8±0.1 kg.d⁻¹ for concentrate. The nutrient intakes estimated for crude protein, ether extract, NDF, structural and non-structural carbohydrates were 1.2, 0.2, 0.8, 1.5, and 0.3 kg.d⁻¹, respectively. The mean eCH₄ was 148.4±30.9 g.d⁻¹, corresponding to 17.4±5.4 g.kg⁻¹ of weight gained. The Pearson correlation coefficient showed a negative association between eCH₄ and TDMI (r=-0.64), suggesting that higher intake, particularly of forage, was associated with lower eCH₄ due to a similar pattern was found with forage intake (r=-0.63), while the relation with concentrate intake was weak (r=-0.16). Although, eCH₄ showed no significant association with feeding variables, it was positively related to eCH₄.DWG⁻¹ (g.kg⁻¹) with r=0.65. This study is the first measurement of methane emission from BON cattle grazing tropical grasses in the Inter Andean Valley of the Antioquia department. It is important to highlight that the emissions observed were lower than other reports for different breeds in the same region, underscoring the BON breed's potential for environmentally sustainable production systems. In addition, future studies could evaluate how variability in heterogeneous pasture, dietary composition, selection of forage and intake behaviour can influence methane emissions under grazing condition.

323 Cumulative emissions of greenhouse gas in agricultural systems in Brazil

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Integrated systems, such as crop-livestock integration (**CLI**), represent sustainable strategies for mitigating greenhouse gas (**GHG**) emissions while enhancing land-use efficiency. CLI involves rotating agricultural crops and pastures in the same area over time, influencing the carbon/nitrogen ratio and subsequently the processes of nitrogen mineralization and immobilization. This dynamic regulates the availability of ammonium and nitrate, which fuel nitrification and denitrification processes directly linked to GHG emissions. However, comparative data on the performance of CLI versus monoculture pastures (MP) and reference areas such as native vegetation (**NV**) remain scarce. This study aimed to evaluate the cumulative emissions of nitrous oxide (**N₂O**), methane (**CH₄**), and carbon dioxide equivalents (**CO₂eq**) in CLI, MP, and NV systems. The treatments included: (A) CLI with soybean (*Glycine max*) planting followed by corn (*Zea mays*) intercropped with Marandu grass (*Urochloa brizantha* cv Marandu); (B) MP with Marandu grass; and (C) NV typical of the Atlantic Forest biome in São Paulo, Brazil. GHG emissions were monitored over two experimental years using the static chamber method. Gas samples were collected weekly during the rainy season and biweekly during the dry season between 9:00 and 10:00 a.m. The chambers remained sealed for 30 minutes, and samples were taken at 0, 15, and 30-minute intervals, then analyzed via gas chromatography. Data were tested for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests, respectively. A one-way analysis of variance was applied to the cumulative emissions, followed by Tukey's test for mean comparisons at a significance level of 5% ($p < 0.05$). Results showed that N₂O emissions ranged from 0.891 to 0.977 kg N₂O ha⁻¹ in year 1 and from 1.007 to 1.381 kg N₂O ha⁻¹ in year 2. Higher emissions were observed in the CLI system, though differences were not significant (year 1: $p = 0.961$; year 2: $p = 0.735$). CH₄ emissions ranged from -0.112 to 2.718 kg CH₄ ha⁻¹ in year 1 and from 0.045 to 2.300 kg CH₄ ha⁻¹ in year 2, with significant differences (year 1: $p = 0.004$; year 2: $p < 0.001$). MP had significantly lower CH₄ emissions than CLI and NV in year 1 and lower than CLI (but not NV) in year 2. CO₂eq emissions ranged from 0.338 to 0.468 t CO₂eq ha⁻¹ in year 1 and from 0.512 to 0.618 t CO₂eq ha⁻¹ in year 2, with no significant differences between systems (year 1: $p = 0.718$; year 2: $p = 0.861$). These findings suggest that CLI can intensify production without increasing GHG emissions compared to reference systems, offering a promising solution for low-carbon agriculture.

328 Seasonality of methane emissions of beef cattle grazing on native sagebrush in Western United States.

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Methane (**CH₄**) emissions from cattle is a significant contributor for the overall agricultural greenhouse gas emissions. Ruminants produce CH₄ primarily through a process called enteric fermentation, which is highly influenced by the nutritional quality of the diet. Literature on CH₄ emissions for extensive production and for grazing cattle is scarce. Therefore, the goal of this study was to quantify CH₄ emissions from cattle raised in the Western U.S., where cows are typically fed during the winter and graze on native rangelands from spring to fall. A total of twenty-two cows (Angus × Hereford) were randomly selected from the Eastern Oregon Agricultural Research Center (**EOARC**) herd, and were acclimated in the spring to Greenfeed Pasture System monitoring units (C-Lock) to measure CH₄ emissions (g/day). During this acclimation period, the cows were maintained as a group and provided meadow hay *ad libitum* (8.5% CP and 56% TDN). Once the cows were acclimated to the monitoring systems, they, along with the monitoring units, were moved to the Northern Great Basin Experimental Range (**NGBER**) in Riley, OR, to assess CH₄ emissions during the grazing season. The monitoring units were strategically placed near water sources and programmed to record up to 8 visits per cow each day, with alfalfa pellets used as an enticement. Throughout the study, cow body weight (**BW**) and body condition scores (**BCS**) were recorded. Additionally, the cows' locations and grazing behavior were tracked using virtual fence collars (Vence, Merck). Preliminary data for CH₄ emissions for the spring season (5 weeks; approximately 3000 emissions records), were analyzed over time as repeated measures, including week, and period of the day (morning, afternoon, evening, and night) with cow as a covariate ($P < 0.001$), using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Statistical significance was defined at $P \leq 0.05$. Preliminary data analysis for the spring season reported effects of week ($P = 0.03$) and period ($P < 0.0001$) for CH₄ emissions. Methane emissions gradually increased over time peaking on week 5 (180 vs. 210 g/cow daily, respectively for week 1 and 5). Interestingly, for the period of the day, CH₄ emissions were the lowest in the morning (6:00 to 11:59 am; 143 g/cow), intermediate in the afternoon (12:00 to 5:59 pm; 193 g/cow), and greatest in the evening (6:00 to 11:59 pm; 221 g/cow) and night (00:00 to 5:59 am; 218 g/cow), with no differences between evening and night ($P = 0.77$). Additionally, from spring to fall, cow BW change was observed ($P = 0.001$). Cows BW fluctuated approximately 24kg (542 vs. 566 kg, respectively, for fall and summer). These preliminary data present a large variation in CH₄ emissions over time and within the day, which should be accounted for when evaluating CH₄ emissions of cattle.

334 Tree carbon sequestration and net GHG emissions on Victorian livestock farms

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Livestock producers must reduce greenhouse gas emissions (**GHGe**) to meet supply chain targets aligned with the Paris Agreement (COP21). In the short term, the most promising method for reducing net farm emissions is through carbon sequestration in woody vegetation. However, the potential for sequestration via tree planting varies widely depending on factors such as planting area, configuration (e.g., shelterbelts vs. blocks), species, establishment and management practices, and local climate and soils. We hypothesised that farms in higher rainfall zones, with a greater proportion of shelterbelts and/or timber species, would achieve higher sequestration rates per hectare and thus greater reductions in net emissions.

To test this, we used a case study approach across four livestock farms in Victoria, Australia. We modelled sequestration potential using FullCAM, the tool used for Australia's national land sector carbon accounting and determined farm emissions (Scope 1, 2 & 3) with the Sheep and Beef Greenhouse Gas Accounting Framework (SB-GAF) calculator. On one property, Jigsaw Farms, we also measured tree growth directly to compare modelled and observed sequestration.

On high-productivity farms (14–18 DSE/ha), modelling showed that proposed plantings covering 4.8%, 5.2%, 11.6%, and 17.2% of the farm area could reduce net farm emissions by 20%, 23%, 33%, and 25% respectively, over 30 years. Two farms achieved net zero emissions temporarily – one for two years and the other for eight. However, net zero was not sustained as tree growth plateaued over time. Shelterbelts had higher sequestration rates per hectare than block plantings. A timber-focused planting did not substantially outperform mixed environmental plantings, likely due to its location in an already high-potential site with a high proportion of shelterbelts. At Jigsaw Farms, estimates of carbon sequestration varied significantly by method. The standard FullCAM model, used for national inventory purposes, underestimated sequestration by 100% to 300% compared to on-site measurements. This suggests that modelled values may be overly conservative for high-productivity systems or well-managed tree plantings.

These findings have several implications. While trees can significantly reduce net farm emissions, they are not a stand-alone solution. The challenge of net emission reduction suggests many farmers will want to keep any carbon credits they earn for their own use rather than selling them. It also highlights the importance of integrating trees for co-benefits, such as productivity gains or ecosystem services. When trees serve multiple roles, they remain valuable even if their carbon benefits diminish over time. Lastly, as market signals for emissions reductions grow stronger, demand for accurate, farm-specific data will increase. This could create tensions between farm-level ("bottom-up") and national-scale ("top-down") accounting approaches.

345 Tier-2 and Tier-1 enteric methane emission factors for pastoral and agro-pastoral cattle systems in Uganda

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Uganda prepared its First Biennial Update Report using the Intergovernmental Panel on Climate Change (IPCC) Tier-1 methodology, which showed that enteric methane (CH₄) emissions had increased from 367 in 2005 to 551 Gg CH₄ in 2015. Over 80% of these emissions were from cattle primarily raised in pastoral and agro-pastoral systems. The 2006 IPCC Guidelines for national greenhouse gas (GHG) inventories recommend using Tier-2 methodology for key source categories which significantly contribute to a country's total GHG emissions. Unlike the Tier-2 methodology which accounts for changes in management, animal performance or mitigation measures, Tier-1 estimates reflect only changes in livestock numbers. However, implementing Tier-2 methodology requires data on average daily feed intake for different animal sub-categories, which are rarely available particularly for grazing livestock. While the Tier-2 guidelines offer net energy equations to estimate feed intake when actual measurements are unavailable, in Uganda, the necessary input data are often unavailable, incomplete or of poor quality. This study aimed to estimate Tier-2 enteric CH₄ emission factors (EFs) for cattle in pastoral and agro-pastoral production systems in Uganda using the IPCC Tier-2 model based on gross energy content of the feed, and compare them with Tier-1 default EFs. The daily gross energy intakes for mature females, mature males, heifers, growing males, and calves sub-categories were estimated during dry and rainy seasons by multiplying DM intake (calculated from faecal output measured using Cr-mordanted fibre alongside DM digestibility determined using acid insoluble ash marker) by gross energy content of the diet. In the IPCC Tier 2 model, the standard 365-day year was adjusted to 182 days for the dry season and 183 days for the rainy season. Tier-2 EFs were then calculated (as sums of dry and rainy season emissions) and compared with IPCC Tier-1 default EF values. Results showed that Tier-2 EFs for "non-dairy" pastoral cattle were 23.3, 27.6, 16.1, 18.1 and 10.6 kg CH₄/head/year for mature females, mature males, heifers, growing males, and calves, respectively. For agro-pastoral production system, EFs for the above sub-categories were 28.7, 30.7, 15.2, 12.6 and 10.5 ("non-dairy"), and 28.4, 43.0, 29.9, 27.0 and 9.6 ("dairy"), respectively. Tier-2 EFs for "non-dairy" were comparable between pastoral and agro-pastoral, but generally lower (18-54%) than those for "dairy". Weighted average EFs (based on sub-category contribution to herd population) for "non-dairy" pastoral (19.1) and agro-pastoral (19.7) cattle were 36.5-38.4% lower than the IPCC Tier-1 default value (31). For "dairy" agro-pastoral cattle, the weighted EF (25.0) was 45.7% lower than the IPCC Tier-1 default value (46). In conclusion, IPCC Tier-1 default EFs overestimate enteric CH₄ emissions from Uganda's pastoral and agro-pastoral cattle. Therefore, by developing country-specific Tier-2 EFs, this research provides a more accurate foundation for national GHG inventories which are essential for designing context-specific mitigation strategies.

372 Emission factor of enteric methane from dairy cows in the Andean region of Colombia

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The use of proprietary emission factors with direct measurements (Tier 3) for enteric methane is important, partly due to the high diversity of the country's production systems. It reduces uncertainty when default emission factors are assumed. Direct measurements result in more accurate, reliable and appropriate data for the design of sustainable and scalable mitigation strategies and techniques. Enabling Colombia to meet its international commitments to reduce emissions. The objective was to determine the emission factor (**EF**) for Holstein dairy cows under grazing conditions on kikuyu (*Cenchrus clandestinus*) pastures in the Andean region of Colombia.

Five dairy cows were located east of the city of Medellín (Santa Elena), Antioquia province, Colombia, at an altitude of 2,200 m, with a temperature of 14.2°C and a rainfall of 2,500 mm/year, in a low montane humid forest (**LMF**) ecological zone. Descriptive statistics were used with daily measurements for six consecutive days. The cows were multiparous (four lactations) Holstein cows with an average live weight (**LW**) of 524 ± 48 kg, 110 ± 15 days in lactation, and an average production of 31.3 L/cow/day. The experimental area was 1.9 ha, divided into nine paddocks of 2,111 m². The pasture was fertilized with 420 kg of N, 50 kg of P₂O₅ and 180 K₂O kg/ha/year. Grazing management used 2111 m² plots, managed with a mobile electric fence with one day of occupation and 42 days of rest. Forage supply was determined using the double sampling technique and canopy height was measured with Sward Stick with 85 measurements/plot. Grazing intake per animal was measured, using chromium oxide as an external marker and indigestible NDF as an internal marker. Feed supplementation was 5.5 kg/cow/day with a commercial concentrate. Milk production per animal/day and its compositional quality were measured. Daily CH₄ emissions were determined using the SF₆ tracer technique. The SF₆ permeation tubes used had permeability rates of 5.4318 ± 0.0426 mg SF₆/day and were deployed in the reticulum of the animals (oral dosing), 20 days before the start of the measurements.

Forage supply was 3699 kg DM/ha, with a forage canopy height of 40 cm. Forage intake under grazing was 16.57 ± 0.72 kg DM/cow/day), with a forage nutritional quality of: 17.7% CP; 65.7% NDF; 27.4% ADF; Lignin 1.98%; OM 91.28% and IVDMD 67.4%. Milk production was 31.6 ± 0.7 L/cow/day, and its compositional quality: protein (3.32%); fat (3.15%); lactose (4.82%) and NUM (18.42 mg/dL).

In conclusion, the enteric methane emissions found were on average 513.9 ± 98.7 g/CH₄/cow/day, with a variation of 19.2% (N=30), which is equivalent to 187.5 Kg/CH₄/cow/year as emission factor with an emissions efficiency of 16.3 g/CH₄/L of milk, allowing to improve the accuracy of the country's enteric methane inventories, and support the baseline for future mitigation programs.

374 Impact of soil treatments on carbon sequestration, carbon stocks, and soil properties

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This study assesses the effects of various fertilization techniques and tillage systems on the physical characteristics, chemical makeup, sequestration of carbon, and carbon stock of soil. The study contrasted four fertilization treatments control, cow dung (**CD**), poultry manure (**PM**), and NPK with three tillage techniques: conventional tillage (**CT**), no-till (**NT**), and minimum tillage (**MT**). The textural class of the soil remained sandy loam throughout all treatments, according to the results, with NT and PM considerably enhancing soil aggregation and structure. Higher levels of total nitrogen (**TN**), organic matter (**OM**), and organic carbon (**OC**) were seen in NT and MT. When it came to improving soil fertility, carbon stock, and general health, PM was the most successful fertilizing technique. According to the study's findings, organic fertilization and reduced tillage improve soil fertility, raise carbon sequestration, and support sustainable farming methods. These results imply that by enhancing carbon storage in agricultural soils, implementing conservation tillage in conjunction with organic fertilization could lessen the effects of climate change.

391 Effectiveness of bioslurry use as fertilizer in enhancing forage production and reducing greenhouse gas emissions

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Livestock farming is vital to many economies in Sub-Saharan Africa, supporting food security and income, but faces feed scarcity and quality. Bioslurry (**BS**), a co-product of anaerobic digestion of manure, is emerging as a sustainable alternative to chemical fertilisers (**NPK**) for growing feed, improving soil health, and reducing greenhouse gas (**GHG**) emissions. The objective of this study was to evaluate BS use as fertilizer on forage yield and quality, soil fertility and GHG (CH_4 , N_2O , and CO_2) emissions from soil. A field experiment was conducted at International Livestock Research Institute (**ILRI**) in Nairobi, Kenya, in a randomized complete block design to evaluate Napier grass production under six treatments: BS, farmyard manure (**FYM**), NPK, 50% BS + 50% NPK, 50% FYM + 50% NPK (total N basis), and control (no fertilizer). Treatments were applied at 100 kg N/ha with four replications. Agronomic data, GHG emissions (using static chamber method), biomass yield and feed quality were monitored from May to August 2024. Soil and climate data were collected. Data for two harvests analyzed as part of a long-term study.

The total dry weight biomass yields for two harvests, on average, were: Control (2.27 t/ha), BS (3.38 t/ha), FYM (2.95 t/ha), NPK (2.69 t/ha), BS + NPK (3.84 t/ha), and FYM + NPK (2.91 t/ha). The application of BS + NPK produced the highest Napier grass yields, with the 1st harvest dry weight yields of 1.37 t/ha and second harvest yields of 2.47 t/ha. Sole NPK application produced lowest yields after the control. Despite lower rainfall during the second harvest, the dry matter yield increased by 55% compared to the first harvest, likely due to the recent establishment of plots. It is anticipated that yields will continue to improve moving forward. The sole BS application resulted in the highest cumulative emissions of N_2O (4.84 kg N_2O -N/ha) and CH_4 (1.65 kg CH_4 -C/ha). In contrast, BS + NPK significantly reduced N_2O emissions (1.96 kg N_2O -N/ha) and acted as a CH_4 sink (-0.12 kg CH_4 -C/ha). The highest net global warming potential (**GWP**) was observed for sole BS (1.33 t CO_2 -eq/ha), while BS + NPK (0.52 t CO_2 -eq/ha) and FYM + NPK (0.57 t CO_2 -eq/ha) showed moderate GWP. This shows the benefits of combining organic and chemical fertilizers for reducing GHG emissions, particularly when focusing only on emissions from forage plots. Yield-scaled emissions further supported combined treatments, with BS + NPK (0.13 t CO_2 -eq/t) and FYM + NPK (0.20 t CO_2 -eq/t) showing lower emissions relative to productivity. BS + NPK consistently acted as a CH_4 sink across both harvests. In conclusion, combining bioslurry and chemical fertilizers provides balanced trade-offs between productivity and environmental impact, making it a more sustainable option than sole BS application. Additional data will be shared during the conference. Future studies involving life cycle analyses of GHG emissions covering the upstream production of bioslurry and downstream impacts of animal feeding would provide a complete picture.

399 Long-term measurement of greenhouse gas emissions and carbon balance of grazing livestock in Argentina

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Most cattle in Argentina are raised on grazing land year-round, and our research aims to explore alternatives for reducing greenhouse gas (**GHG**) emissions while improving the overall carbon balance (**CB**) of diverse pasture-based livestock production systems. To achieve this, case-study farms across diverse agroclimatic regions, representing the country's main production systems, are being monitored under long-term studies. These sites include, a tropical silvopastoral beef cattle (Misiones), two sub-tropical native silvopastoral beef cattle (Salta and Tucumán), two sub-tropical cow-calf systems located in wet (Corrientes) and dry (La Rioja) regions, a temperate intensive beef-crop production (Buenos Aires), and a dry sub-Antarctic extensive sheep production (Patagonia). This study is part of a broader national initiative to monitor GHG emissions and CB in pasture-based livestock systems over time, aiming to generate robust data for Argentina's diverse agroecological regions. At two of the abovementioned case-study farms, the CB was estimated as the difference between the C gained through pasture net primary production (**NPP**) and any eventual C import (e.g., as feeds or hay), minus the C lost as CO₂ from animal and soil respiration, as methane (**CH₄**) from enteric fermentation, and as C exported meat and exported hay. The NPP was computed as the sum of aerial NPP (**ANPP**) and belowground NPP (**BNPP**). The ANPP was assessed through pre- and post-grazing shoot biomass measurements, while the BNPP was estimated after the ANPP and assumed root/shoot partition coefficients. Soil respiration C losses were estimated according to the AMG soil model. Animal GHG emissions were measured with one GreenFeed (**GF**) unit each, installed next to the water trough and permanently accessible for voluntary visits. In an intensive beef backgrounding system (Buenos Aires), emissions were monitored for 295 days, from May 26, 2024, to March 17, 2025. The system consisted of 77 Angus replacement heifers (199 ± 19 kg initial LW, 636 ± 76 g.day⁻¹ ADG, mean $\pm$ SD) grazing 22 ha of alfalfa and tall fescue pasture under a 3-day rotational management. Hay from the same pasture was offered during the winter. A total of 36 animals were successfully recorded. Finalized GF data indicate that enteric GHG emissions averaged $4,884 \pm 1,310$ and 137 ± 45 g.animal⁻¹.day⁻¹ of CO₂ and CH₄, respectively. The second production system consisted of a breeding cow-calf operation (Corrientes). Sixty mature beef cows (410 ± 45 kg mean LW, 540 ± 250 g.day⁻¹ ADG) grazing 90 ha of a native grassland were assigned to a trial for 52 days, from Nov 19, 2024, to Jan 10, 2025. A total of 26 animals were measured with another GF unit. Finalized data indicated an average of $6,914 \pm 1,243$ and 210 ± 69 g.animal⁻¹.day⁻¹ of CO₂ and CH₄, respectively. Results from soil respiration, NPP, forage quality and intake of both production systems are underway. Differences in animal emissions between systems seem to be related to the type of animal and forage quality. These results, together with the CB of the first year, will be presented at the Conference.

403 Comparison of contemporary grazing cattle and bison GHG emissions in the Southern Great Plains

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In recent years, there has been interest, both within the U.S. and abroad, to examine how contemporary pasture production methods compare to “natural” or historic emissions on these landscapes from other herbivores. To our knowledge, the emissions generated from intensive seasonal grazing approach have not been compared to a “natural” system or historic emissions from migrating bison. The objective of this analysis was to compare the enteric methane (**CH₄**) and manure greenhouse gas (**GHG**) emissions from contemporary grazing cattle production with bison grazing, both modern and historical. The data sets used in this analysis were derived from existing research and conservation properties located outside Manhattan, KS (USA) that are home to stocker cattle, commercial cow-calf production, and grazing bison. For stocker cattle, 10 years of animal production data (2007 – 2016) from both season-long stocking (**SLS**, grazing 156 d) and intensive early stocking system (**IES**; 76 grazing d and 2x stocking density) were used for GHG calculations. Enteric CH₄ production was estimated using IPCC tier 2 methodology and manure CH₄ and direct nitrous oxide (**N₂O**) production using IPCC tier 2 and tier 3, respectively. Cow-calf system (**CCS**) GHG production was estimated using a stocking rate of 3.25 ha/cow under a year-long grazing plan, and intake was assumed to be 2.2% of the cow's body weight. Modern grazing bison (**MGB**) GHG production was estimated using population and forage intake assessments from the Konza Prairie Preserve where the bison are currently housed. Historic bison (**HGB**) enteric CH₄ estimates were calculated using the established forage intake rate of the Konza, a stocking density of 0.15 ha/animal and with the assumption that only 13% of grassland was used by the plains bison in a given year, assuming unrestricted migration patterns. Contemporary CCS resulted in a lower annual enteric CH₄ production than MGB (408.9 vs. 610.7 kg CO₂-eq./ha/yr). The IES production system had the lowest annual emission rate of the 4 contemporary production systems on a yearly basis at 365.3 kg CO₂-eq./ha/yr, followed by SLS and CCS. These estimates for IEE were similar to HGB enteric CH₄ production on a yearly basis, estimated at 352.5 kg CO₂-eq./ha/yr. Manure GHG emission rate of the contemporary systems had higher emissions than both MGB and HGB. Within the contemporary systems, CCS had the lowest emission rate while IES, SLS and MGB had the highest GHG estimates (75.0, 98.0, 98.65 and 103.6 kg CO₂-eq./ha/yr, respectively). HGB manure emissions had the lowest annual manure emission rate at 59.8 CO₂-eq./ha/yr. These results imply that the historic grazing baseline of this grassland system is similar to contemporary grazing cattle in the Great Plains region, which should be considered in the larger discussion on climate policy and shifting agricultural systems. Further work should quantify the grazed vs. ungrazed landscape to better understand the tradeoffs between modern land utilization and historic land use by wild herbivores.

411 Factors affecting GHG emissions in agricultural systems in the Atlantic forest biome in Brazil

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Pastures are significant sinks of greenhouse gases (**GHG**). However, intensification through increased animal stocking rates and/or the use of nitrogen fertilizers can increase nitrous oxide (**N₂O**) production by denitrifying microorganisms. A promising solution is the adoption of integrated crop-livestock production systems (**ICLs**), in which agricultural residues, such as straw, influence the carbon/nitrogen (**C/N**) ratio of the system. These factors affect the dynamics of nitrogen mineralization and immobilization, as well as the diversity and abundance of microorganisms involved in **N₂O** and methane (**CH₄**) emissions and consumption. This study aimed to evaluate how environmental and microbiological variables influence **N₂O** and **CH₄** emissions in integrated systems, comparing them with monoculture pasture (**PM**) and native vegetation (**NV**) of the Brazilian Atlantic Forest biome. Three treatments were tested: (A) ICLs with soybean followed by corn intercropped with marandu grass; (B) PM with marandu grass; and (C) NV typical of the Brazilian Atlantic Forest biome. GHG emissions were monitored over two years using static chambers, with weekly sampling during the rainy season and biweekly collection in the dry season. The chambers remained sealed for 30 min, and gas samples were collected at 0, 15, and 30 min and analyzed by gas chromatography. Principal Component Analysis (**PCA**) was used to explore how environmental and microbiological variables drive cumulative **N₂O** and **CH₄** emissions in the different systems. PC₁ (35.3%) showed positive correlation with soil temperature ($r = 0.85$, $p < 0.001$), air temperature ($r = 0.83$, $p < 0.001$) and water-filled pore space (**WFPS**) ($r = 0.68$, $p < 0.001$), but negative correlation with **NH₄⁺** ($r = -0.77$, $p < 0.001$) and **NO₃⁻** ($r = -0.72$, $p < 0.001$) concentrations. PC₂ (21.1%) showed positive correlations for AOA ($r = 0.82$, $p < 0.001$) and denitrifying enzymes (**nirK**, **nirS**), while PC₃ (12.1%) demonstrated a positive relationship between **N₂O** ($r = -0.69$, $p < 0.001$) and **CH₄** ($r = -0.61$, $p < 0.001$) production. When analyzing the bi-plot, we observed temporal variations in the behavior of land use systems in relation to the PC₁ and PC₂. In PC₁, all systems showed a positive relationship in the first year of evaluation, indicating an association with variables located on the right side of the graph, such as soil temperature, **NO₃⁻**, **NH₄⁺**, **nirK** and **nirS**. However, in the second year, the same systems had an inverse behavior, approaching variables such as **N₂O**, **CH₄**, **nosZ**, AOA and **WFPS**, which suggests a change in the dynamics of biogeochemical processes over time. In PC₂, the systems maintained similar behavior between years, evidencing a certain stability in the contribution of this component to the differentiation of land uses. The exception was the ILP system, which showed opposite behavior between the years of evaluation. This shift suggests a relevant change in the factors that make up PC₂ for the ILP system, possibly related to temperature variability and soil moisture. The ILP due to crop successions proved to be more susceptible to changes in precipitation and temperature gradient, since crop rotation reduced vegetation cover, resulting in lower moisture retention and higher soil temperature.

414 Nitrogen cycling and losses in *Urochloa* pastures: legume inclusion and phosphorus effects in Amazon soils

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In the Amazon region of Colombia, pastures originated from deforestation during the mid-20th century and have since then been managed as grass monocultures without nitrogen (**N**) fertilization. These systems often experience N deficiencies, limiting productivity and ecosystem sustainability.

The integration of legumes into pastures has been proposed as a strategy that may alleviate N deficiencies via biological N fixation and N transfer to grasses. However, on-farm measurements from our project revealed higher soil mineral N concentrations in grass-legume pastures compared to grass-alone systems. This increased soil mineral N was associated with elevated soil nitrification rates, suggesting a greater potential for N₂O emissions and nitrate (**NO₃⁻**) leaching, raising environmental concerns.

To further investigate this, we conducted a soil column experiment at the Alliance Bioversity-CIAT research station in Palmira, Colombia. The experiment evaluated the effects of pasture composition (grass species and legume presence) and phosphorus (**P**) fertilization on N dynamics, using repacked soil profiles (0–50 cm) in experimental units of 50 kg of dry soil equivalent each. Grass species included *Urochloa humidicola* CIAT 679, known for its biological nitrification inhibition (**BNI**) properties, and *Urochloa brizantha* CIAT 6294, with the legume *Arachis pintoi* CIAT 17434. P fertilization was applied at two levels (0 and 40 kg ha⁻¹).

Preliminary results from the column experiment indicate on average 13% higher N concentration in grasses associated with legumes, and 18% higher plant aboveground biomass production when pastures are fertilized with P, representing two mechanisms for increasing plant N uptake.

As a next step, in June 2025 we will quantify N₂O emissions, using a portable multigas analyzer, analyze δ¹⁵N and δ¹⁸O isotopes of N₂O to identify emission pathways, and measure **NO₃⁻** leaching through leachate samples and soil pore water collected at different depths. Concurrently, we will monitor plant biomass productivity, biological N fixation, and the composition of mineral N in the soil solution. These measurements, which will be presented in October 2025 at the GGAA conference will provide critical insights into the mechanisms driving N dynamics in these systems.

Our research aims to inform sustainable pasture management strategies in the Amazon by balancing productivity gains with environmental sustainability. If P fertilization proves effective as an N plant uptake enhancer, it could offer a viable mitigation strategy for reducing N₂O emissions in pastures with limited BNI capacity. This work contributes to the broader goal of reducing the environmental footprint of livestock systems while maintaining their productivity and ecosystem services.

420 Low-methane forages: Discovery, development and deployment in the Global South

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Food systems are responsible for approximately 60% of anthropogenic methane (CH₄) emissions; livestock contributes half. Reducing enteric methane emissions is a crucial strategy in addressing climate change as CH₄ is a more potent greenhouse gas than carbon dioxide (CO₂). One way to reduce CH₄ emissions from the digestive process of ruminant animals is by identifying and developing low-methane forages (LMF). Forages, including browses, legumes and grasses containing anti-methanogenic compounds (AMC), such as tannins, saponins, flavonoids, H₂-sinks, or methanogenesis inhibitors, constitute LMF. These offer a potentially cost-effective solution for reducing CH₄ production from livestock systems, whilst maintaining or improving productivity. An international collaborative project among the three CGIAR Centers (Alliance Bioversity-CIAT, ICARDA, ILRI) and two specialized research laboratories (AgResearch, New Zealand and; Universidad Javeriana, Colombia) is focusing on three key areas: (i) identifying and promoting the use of LMF into ruminant production systems in the Global South; (ii) adding a CH₄ emission-reducing trait into widely used forage cultivars using conventional or precision breeding (gene-editing) approaches; and (iii) deploying LMF into forage-based livestock (ruminant) systems in the Global South. Results from a pilot study on *in vitro* gas and CH₄ production identified several promising forage accessions with low to moderate CH₄ production relative to dry matter digestibility. In 2024, a total of 407 forage materials were assembled by the three CGIAR Centers and grown in the field for *in vitro* screening. Initial metabolomic analyses have advanced toward developing and deploying an HPLC-MS data analysis pipeline to streamline the identification, and annotation of metabolites. A protocol for genetic transformation of forage grass *Urochloa humidicola* (CIAT 16888) has been developed to facilitate gene-editing. Rumen microbiome studies are envisaged to understand rumen microbial ecology. At the Colombia campus of the Alliance, 16 respiration chambers were constructed, eight of them tailored for large ruminants and eight for small ruminants. This specialized infrastructure will play a crucial role in validating *in vivo* any promising forages identified through *in vitro* screening, to support their subsequent scaling for broader applications. At ICARDA's Marchouch station in Morocco, significant progress has been made in developing a similar infrastructure for small ruminants. The ILRI team is using the existing respiration chambers for *in vivo* CH₄ measurements while complementing these measurements with sulfur hexafluoride (SF₆) methodology. Ongoing efforts include developing LMF-specific emission factors, designing cost-effective MRV systems, and deploying climate finance mechanisms aimed at streamlining broad adoption of LMF. The LMF project contributes to the aims of Enteric Fermentation Research and Development Accelerator of the Global Methane Hub. These initiatives represent significant progress in aligning data-driven approaches with practical tools to improve greenhouse gas accounting, enhance livestock productivity, and support sustainable land management strategies under diverse agroecological contexts.

425 Effect of fertilization types on greenhouse gas emissions in irrigated rice production in Burkina Faso

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Addressing the dual challenges of food security and environmental protection requires optimizing crop management to achieve high yields while reducing greenhouse gas (GHG) emissions. Farmers' management practices can significantly influence GHG emissions; however, few studies have comprehensively quantified the effects of local fertilization on soil GHG emissions to support climate-smart agricultural strategies. This study aimed to evaluate the impact of local fertilization on GHG emissions in irrigated rice production. A factorial experimental design with pairwise comparisons was applied, incorporating three fertilization treatments with two replications each: **T1** (chemical fertilization with NPK + urea), **T2** (mixed fertilization with manure + NPK + urea), and **T0** (no fertilization). Soil emissions of CH₄, CO₂, and N₂O were monitored using a static chamber gas system during rice growing cycle. Each replication accounted six chambers in which gas were sampled four times at 0, 10, 20 and 30 mn at each measuring day. Gas samples were analyzed at the University Joseph Ki-Zerbo using gas chromatography (GC 8610 C, SRI, USA). During the rice growing season, CH₄ emissions ranged from 24.87 ± 26.43 to 142.93 ± 25.8 kg ha⁻¹, with significant differences among treatments ($P < 0.001$). The highest CH₄ emissions (142.93 ± 25.8 kg CH₄-C ha⁻¹) were observed under T1 following to mixed fertilization (T2), and lowest (109.94 ± 30.52 kg CH₄-C ha⁻¹) under (T2). N₂O emissions increased significantly under both fertilization treatments, with T1 (3.05 ± 0.36 kg N₂O-N ha⁻¹) and T2 (2.78 ± 0.10 kg N₂O-N ha⁻¹) exceeding the no-fertilizer treatment by 115.18% and 95.93%, respectively. No significant effect of fertilization was observed on CO₂ emissions. Regarding emission factor (EF), only CH₄ EF was significantly affected ($P < 0.05$), with the highest value (1.19 ± 0.15 kg ha⁻¹ day⁻¹) recorded T2. T1 and T2 increased global warming potential (GWP) by 98.5% and 136.2%, respectively, with CH₄ emissions contributing 50.02% of the total GWP. Chemical fertilization in irrigated rice production increases CH₄ and N₂O emissions. Therefore, mixture fertilization with low rate of chemical fertilizers may present a promising strategy for reducing GHG emissions in rice production. Further investigation is needed to define appropriate mixture fertilization meeting both good rice yields and climate mitigation.

429 Carbon footprint of rearing Nellore young bulls as affected by pasture nitrogen fertilization sources

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Beef cattle production is one of the main economic activities in Brazil, and it has been associated with climate change. Beef cattle production is pasture-based on extensive systems. Nitrogen fertilization is one of the options to achieve sustainable intensification of beef cattle production. The use of different sources can influence both productivity and emissions as they have different characteristics. This study was conducted at the UNESP campus in Jaboticabal, São Paulo, Brazil, to evaluate the carbon footprint (CO₂eq/kg of live weight) of beef cattle rearing in palisade grass (*Urochloa brizantha* R. D. Webster cv. Marandu) during the rainy season of 2023. Pastures were managed under continuous stocking with a variable stocking rate; following the put-and-take method, briefly, animals were put and removed to the paddock to maintain 25 cm of height and at a grazing utilization efficiency of 50%. The stocking rate was adjusted using put-and-take animals. Previous studies showed that under these experimental conditions, 25 cm of height promotes 95% light interception, which allows the achievement of maximum net forage accumulation with high leaf production and low senescence, resulting in high-quality forage allowance that may increase animal performance. Forty-eight Nellore bulls, with an average initial body weight of 280± kg (p= 0,81), were used for performance evaluation, in addition to the regulators used for pasture height control in the rearing phase. Animals were distributed in 4 treatments with 3 replications (paddocks -1ha): 1- Control (Without application of nitrogen fertilizers), 2- Fertilization with Urea, 3- Fertilization with Ammonium Sulfate, and 4- Fertilization with Ammonium Nitrate. To calculate the carbon footprint, in CO₂eq per kilogram of live weight, Embrapa's Low Carbon Livestock Calculator was used, based on the IPCC Tier 2 model (IPCC, 2019), which follows the FAO/LEAP guidelines (FAO, 2016), meets the requirements of ISO 14.044 (ISO, 2006) and the ICVCalc food library (Matsuura et al., 2022). The calculator estimates the emissions from animals (CH₄ enteric, CH₄, and N₂O from manure, emissions from feed and fertilizers). Average daily gain (p= 0,061) was affected by fertilizers, the highest values were observed in animals kept on pastures fertilized with ammonium nitrate (average of 1.0± kg/day). The average daily gains of treatment fertilized with ammonium sulfate and urea were, respectively, 0.950± and 0.933± kg/animal/day. The lowest gain was recorded in animals raised on pastures without nitrogen fertilization, averaging 0.743± kg/day. Compared to the no-nitrogen fertilized pasture, a 2.4% reduction in the carbon footprint of animals grazing pastures fertilized with urea was observed. The CF per treatment was 1- 3.137; 2- 3.062; 3- 3.222; 4- 3.218 kg of CO₂ eq/kg LW. Fertilization of pastures with nitrogen fertilizer can be a viable alternative for intensifying cattle production systems on pasture. The urea source presented the lowest emission intensity among the fertilizers tested, even when compared to the treatment without nitrogen fertilizer, with a reduction in CO₂ eq emissions per kg of live weight.

437 Seasonal variation in greenhouse gas emissions across land use types in West Africa semi-arid conditions

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Measuring greenhouse gas emissions in different land use in West Africa is essential for improving regional climate stimulation models and developing mitigation strategies. However, the lack of field measurement data on greenhouse gas emissions makes climate predictions difficult. This study aimed to assess the effect of land use and land cover on the temporal dynamics of CO₂, CH₄ and N₂O fluxes. Gases were sampled twice a month in twelve plots equally distributed between three land use types (croplands, rangelands and forests) in Burkina Faso using the static chamber method from February 2024 to January 2025. Six chambers were installed per plot (1000 m²) including three under tree canopy and three outside tree canopy for accounting the effect land cover on gas fluxes. For each measuring day gases were sampled four times at 0, 10, 20 and 30 min and transported to University Joseph Ki-Zerbo for analysis using a gas chromatograph (GC 8610 C, SRI, USA). The results showed that land use had significant effect on the annual cumulative fluxes of GHG ($p < 0.001$). The highest fluxes were recorded in 36 688.94 kg C ha⁻¹ yr⁻¹ in rangeland and the lowest in 32 365.75 kg C ha⁻¹ yr⁻¹ in cropland. Regarding temporal variation, only CO₂ fluxes showed significant variations ($P < 0.001$) over the months of measurement in all land use types. In addition, CO₂ flux showed significant seasonal variation ($P < 0.001$), with higher emissions during the rainy season compared with the dry season. CO₂ fluxes observed during the rainy season were 466.1 ± 34.35 mg C m² h⁻¹ in croplands, 496.41 ± 24.2 mg C m² h⁻¹ in rangelands and 459.72 ± 24.7 mg C m² h⁻¹ in forests. In croplands and forests, maximum CH₄ fluxes were observed during the rainy season, while in rangelands they were highest in the dry season. For N₂O, the greatest fluxes were recorded in croplands and rangelands during the rainy season, while the maximum in forests was noted during the dry season. Results also indicated that land cover has significant influence only on CO₂ fluxes in all land use types ($p < 0.001$). Under-canopy fluxes were 464.73 ± 32.85 mg C m² h⁻¹ in croplands, 455.76 ± 27.13 mg C m² h⁻¹ in rangelands and 423.3 ± 20.44 mg C m² h⁻¹ in forests; in comparison, inter-canopy fluxes were 271.62 ± 13.27 mg C m² h⁻¹, 377.73 ± 23.85 mg C m² h⁻¹ and 366.64 ± 24.32 mg C m² h⁻¹ respectively. In contrast, the influence of canopy on CH₄ and N₂O fluxes was found to be insignificant for all land use types. This study highlighted that land use; land cover and climate conditions are key ecological factors affecting greenhouse gas emissions in West Africa agricultural landscape. Our findings can be used to improve greenhouse gas inventory, mitigation strategies and climate models for the region.

438 How nitrogen fertilizers affect nitrous oxide emissions in maize production in West Africa

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Food demand is increasing with population growth and it is expected to reach up 70% by 2050 in Africa. To meet this demand, agricultural intensification with huge use of synthetic fertilizers is widely practiced resulting in significant emissions of greenhouse gases (**GHG**), especially nitrous oxide emissions. The great challenge is how to increase food production to reach food security by minimizing related GHG emissions. The lack of field measurement on emissions from fertilizers in main food crops as maize limits mitigation actions designing. The objectives of this study were to: (i) assess the effect of different fertilization types on nitrous oxide (**N₂O**) emissions and (ii) calculate associated emission factors.

Gas measurements were carried out in maize field under an experimental design including five fertilization types: mixture fertilization [5 t.ha⁻¹ manure (26.5 kg N) + 200 kg.ha⁻¹ NPK (30 kg N)], local practices [3.6 t.ha⁻¹ manure (19.08 kg N) + 100 kg.ha⁻¹ NPK (15 kg N)], manure only (5 t.ha⁻¹), NPK only (200 kg.ha⁻¹) and control (no fertilization). Gases were sampled from 9 a.m to 3 p.m using static chamber system one day before fertilization (baseline), seven days after fertilization and weekly until harvesting time. Chambers were closed until 20 min before starting gas sampling, which was done four times at 10 min intervals, resulting in 3,600 gas samples collected. The study was conducted in 2023 in the Sudanian zone of Burkina Faso with annual rainfall ranged from 900 to 1100 mm. Gas samples were analyzed at the University Joseph Ki-Zerbo using a gas chromatograph (GC 8610 C, SRI, USA).

N₂O emissions were significantly affected by fertilizers application ($P < 0.001$). The highest cumulative emissions (2.86 kg N₂O-N ha⁻¹) were recorded under mixture fertilization and the lowest in control plots (1.93 ± 0.11 kg N₂O-N ha⁻¹). Mixture fertilization increased N₂O emissions by 32.37%. Regarding N₂O emission factors (**EFs**), NPK application showed a high value (0.37 ± 0.05 %), approximately 63 % lower than the default value recommended by the Intergovernmental Panel on Climate Change (**IPCC**). Yield-scaled N₂O emissions **YSNE** were ranged from 0.98 to 1.34 g N₂O-N kg⁻¹ grain with no significant differences between treatments ($P = 0.074$).

Mixture fertilization is an important source of N₂O emissions in maize production. Therefore, reducing synthetic nitrogen amendment when using mixture fertilization with manure is strongly recommended to mitigate global warming. Our findings will help to improve GHG inventories in agricultural sector and support the development of Nationally Determined contribution (**NDC**). Further investigation is highly needed to find suitable combining of fertilizers ensuring both emission reducing and good maize production.

471 Manure distribution and soil water content controls N₂O emissions and sources

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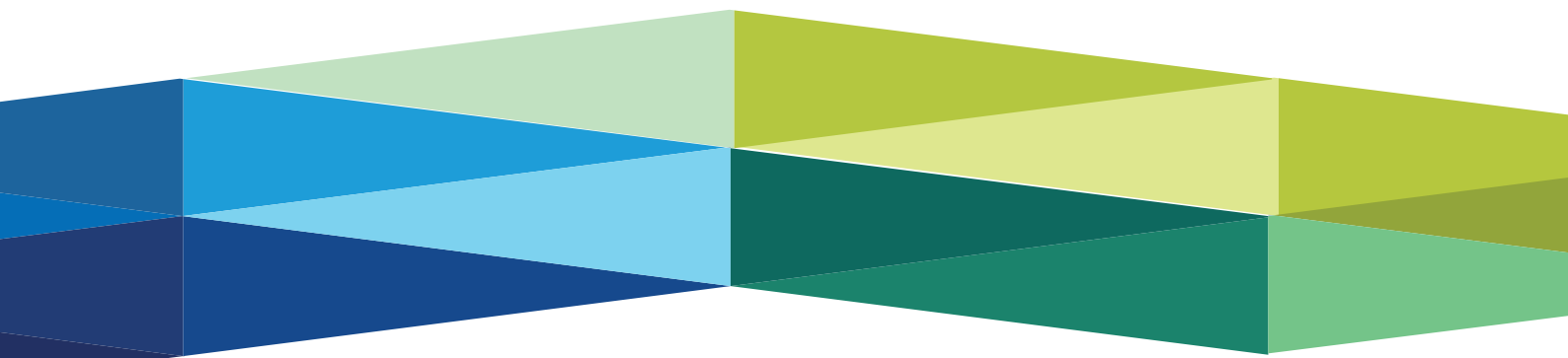
Recycling animal manure to agricultural soils is essential for maintaining soil fertility for crop production but has also been documented as a potential driver of N₂O emissions. In efforts to mitigate emissions, understanding the complex interactions between manure and soil is critical. A 28-day incubation study was conducted to investigate the effects of cattle slurry distribution at three soil matric potentials (ψ_m), i.e., -30, -50, and -100 hPa. Each ψ_m was combined with three manure treatments: i.e., a homogenous mixture of slurry and soil, cattle slurry sandwiched between two soil phases, and an unamended control. Cattle slurry was added at a rate corresponding to 100 kg TN ha⁻¹. To investigate sources of N₂O in each of the treatments, 10 atoms% K¹⁵NO₃ was added to the soil during the experimental set-up (final concentration: c. 20 mg NO₃-N kg⁻¹ dry wt. soil). The results showed an increasing trend of N₂O emissions with increase ψ_m . Irrespective of ψ_m , the trend in N₂O emissions was slurry mixed > slurry as layer > control, with the highest emissions observed on day 1 and a decreasing trend during the incubation. By far the highest N₂O emission rates were observed with the homogenous mixture of cattle slurry and soil at -30 hPa, corresponding to 67% WFPS. The mineral N dynamics as well as the ¹⁵N enrichment of N₂O indicated that denitrification was the dominant source of N₂O with up to 81% of emissions in the mixed-homogenous treatment at -30 hPa, and that at -50 and -100 hPa aerobic conditions dominated and nitrification was the main source of N₂O. The intimate contact between manure C and soil NO₃⁻ probably enhanced N₂O emissions with homogeneous mixing of soil and cattle slurry, whereas NO₃⁻ supply may have limited emissions when the slurry was applied as a discrete layer. We conclude that assumptions about slurry distribution in soil are critical for the prediction of N₂O emissions and effects of soil water content.



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Theme 6: Circular economy



6 Farm-to-Fork in Magical Kenya: Impacts on satisfaction, seed systems, and sustainability

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This mixed-methods study investigates the integration of Farm-to-Fork (F2F) practices within Kenya's hospitality industry, framed within the context of "Magical Kenya," and examines its effects on customer satisfaction, economic benefits for farmers, logistical challenges, and the role of government policies. The research employs a comprehensive approach: a survey of 50 restaurant and hotel managers assesses the extent of local sourcing and its perceived impacts, while interviews with 25 farmers reveal their experiences and challenges. Additionally, a customer satisfaction survey of 300 patrons measures perceptions of food quality and preferences for local sourcing. A policy review further explores the regulatory environment affecting F2F practices.

Findings indicate that integrating F2F practices significantly enhances customer satisfaction, with patrons linking local sourcing to superior freshness, taste, and authenticity. Despite facing logistical challenges such as inconsistent supply and limited infrastructure, establishments with robust local sourcing report long-term reductions in food costs. Farmers benefit from increased income but identify a need for enhanced government support in areas like storage and quality control. The policy review highlights gaps in the national framework that connect agriculture and tourism, which impedes the full potential of F2F models.

This study contributes valuable insights into F2F practices in Kenya's hospitality sector, emphasizing both the benefits and challenges. It underscores the importance of addressing logistical barriers and strengthening policy frameworks to foster sustainable local sourcing. The findings offer actionable recommendations for hospitality businesses, policymakers, and farmers, aiming to create a more sustainable and economically viable food system within Kenya's tourism industry, thus enhancing its "Magical Kenya" appeal.

25 Reducing Methane Emissions and Enhancing Dairy Farm Performance: Insights from Norway

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Dairy farming is a key contributor to global greenhouse gas (GHG) emissions, with methane (CH₄) the dominant gas from enteric fermentation and manure management (Gerber et al., 2013). As climate change concerns intensify, improving dairy production's environmental sustainability has become a priority. The circular economy (CE) has emerged as a critical framework for enhancing resource efficiency while minimizing waste and emissions (Ellen MacArthur Foundation, 2015). In dairy farming, circularity involves optimizing resource use, closing nutrient loops, and reducing environmental externalities. These circular strategies contribute to eco-efficiency by ensuring production processes achieve higher outputs with lower resource consumption and environmental impact (Schader et al., 2016). Eco-efficiency, defined as producing economic value while minimizing environmental harm (OECD, 1998), is a crucial metric for assessing dairy farm sustainability. By integrating circular practices, farms can improve eco-efficiency through targeted interventions such as feed optimization, precision livestock farming, and manure management innovations. Despite the theoretical benefits of circularity, its practical adoption in dairy farming varies due to economic, social, and institutional factors. While some Norwegian dairy farms have implemented manure separation, biogas recovery, and optimized feeding strategies, the extent to which these practices have been adopted across the sector remains unclear. Socioeconomic factors such as farm size, land tenure, financial constraints, and access to government incentives play a crucial role in determining whether farmers integrate circular approaches into their production systems (Läpple & Thorne, 2019). Thus, understanding the interplay between circularity, eco-efficiency, and farm performance is essential for designing policies that drive sustainable dairy production.

This study assesses the performance of Norwegian dairy farms over three decades (1991–2020) through the lens of eco-efficiency and circular economy principles. Using a parametric model and a panel dataset of 692 dairy farms, we analyze the impact of circular strategies on CH₄ emissions and resource efficiency. Methane emissions were estimated based on the Intergovernmental Panel on Climate Change (IPCC) Tier 2 methodology, incorporating country-specific data on dairy cattle diet and production. Circular practices, such as optimizing feed efficiency to lower enteric fermentation and enhancing manure management through anaerobic digestion and nutrient recycling, were examined as potential mitigation strategies. However, due to data limitations, the extent of circular strategy implementation across farms remains uncertain.

Our results show an average eco-efficiency score of 0.95, indicating a potential 5% reduction in resource use and CH₄ emissions without compromising productivity. While circular practices enhance environmental and economic sustainability, adoption depends on socio-economic factors such as land tenure, farm experience, and subsidies. Financial constraints, including high debt-to-asset ratios, were linked to lower farm performance, limiting investment in circular solutions. These findings highlight the need for policies promoting circularity through knowledge-sharing, financial incentives, and manure management. Future research should examine nitrogen emissions and biodiversity to develop a comprehensive sustainability framework. By identifying pathways to reduce GHG emissions while maintaining productivity, this study provides insights for policymakers and industry stakeholders seeking to enhance circularity in Norwegian dairy farming and beyond.

36 Advancing Circular Economy Strategies for Sustainable Livestock Systems: Innovations in Greenhouse Gas Mitigation

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The livestock industry is responsible for a significant share of greenhouse gas (GHG) emissions, predominantly methane (CH₄) from enteric fermentation and manure management, and nitrous oxide (N₂O) from feed production. Circular economy principles, emphasizing resource efficiency and waste valorization, offer a sustainable pathway to mitigate these emissions. This study investigates the effects of integrating anaerobic digestion, waste valorization, and alternative feeds on GHG emissions and nutrient recycling efficiency in livestock systems.

The study employed a randomized complete block design (RCBD) to test the effectiveness of three circular economy interventions: (1) Anaerobic Digestion (**AD**): Recycling manure into biogas and bio fertilizers, (2) Waste Valorization (**WV**): Feeding livestock with agricultural by-products, including crop residues, (3) Renewable Feeds (**RF**): Incorporation of algae-based feeds and insect meal as dietary substitutes. Each treatment was replicated across three livestock systems: intensive dairy farms, smallholder beef farms, and mixed crop-livestock systems. A total of 45 experimental units (15 per system) were monitored for six months. Control groups (conventional practices) were included for comparison. Measurements included: GHG Emissions: CH₄ and N₂O emissions quantified using a portable gas analyzer and chamber-based flux measurement methods; Nutrient Recycling Efficiency: Assessed by analyzing the nutrient content (nitrogen, phosphorus) in bio fertilizers and soil following application; Feed Efficiency: Evaluated by feed conversion ratio (FCR) and animal weight gain.

The collected data were subjected to rigorous statistical analysis: (1) Analysis of Variance (**ANOVA**): A two-way ANOVA was performed to determine the effects of treatments and livestock systems on GHG emissions, nutrient recycling efficiency, and feed efficiency, (2) Post-Hoc Testing: Tukey's Honest Significant Difference (HSD) test was used for pairwise comparisons, (3) Regression Analysis: Relationships between nutrient recycling, feed efficiency, and GHG emission reductions were explored using multiple linear regression, (4) Normality and Homoscedasticity: Shapiro-Wilk and Levene's tests were applied to ensure data met ANOVA assumptions, and (5) All statistical analyses were conducted using R software (v4.2.0), with a significance threshold of $p < 0.05$.

Anaerobic digestion achieved a 47% reduction in CH₄ emissions ($p = 0.001$), while bio fertilizers enhanced crop yields by 18% compared to synthetic fertilizers ($p = 0.004$). Waste valorization led to a 35% decrease in feed-related CO₂-equivalent emissions ($p = 0.003$). Algae-based feeds and insect meal reduced enteric CH₄ emissions by 26% ($p = 0.002$) while improving FCR by 15%.

Regression analysis revealed strong correlations ($r > 0.85$) between nutrient recycling efficiency and GHG reductions. Post-hoc testing confirmed significant differences between treatments and control groups across all metrics. This study demonstrates that circular economy strategies significantly reduce GHG emissions while enhancing nutrient recycling and feed efficiency. Anaerobic digestion, waste valorization, and renewable feeds offer practical solutions for sustainable livestock systems. These findings provide evidence-based recommendations for scaling circular economy interventions to meet global climate and sustainability goals.

103 Indirect method of global warming assessment via plasma nitrogen isotopic discrimination in small ruminants

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Ruminant livestock contribute significantly to methane (**CH₄**) emissions, but the role of nitrogen excretion (**UN**) in this context is often overlooked. Both the proportion of N excreted in the urine to N intake (**NI**; UN/NI) and the N contained in manure (manure-N) are crucial in driving ammonia (**NH₃**) emissions, which indirectly influence global warming. Although NH₃ is not a direct greenhouse gas, its volatilization contributes to the production of nitrous oxide, a potent greenhouse gas with a global warming potential approximately 300 times greater than carbon dioxide. This cascade effect exacerbates environmental degradation and climate change. Effective N management is therefore essential for mitigating both the direct and indirect climate impacts associated with ruminant livestock, including CH₄ emissions. Recent studies (e.g., Cheng et al., 2014; Cantalapiedra-Hijar et al., 2020) have demonstrated the potential to plasma-feed N isotopic discrimination (plasma $\Delta^{15}\text{N}$; plasma $\delta^{15}\text{N}$ – feed $\delta^{15}\text{N}$) to predict N excretion in large ruminants. This study explored the relationship between plasma $\Delta^{15}\text{N}$ and both UN/NI and manure-N in small ruminants using linear and restricted maximum likelihood (**REML**) analysis. Treatment means from four studies reporting UN/NI (g/g), manure-N (g/d), and plasma $\Delta^{15}\text{N}$ (‰) from 62 dry sheep (n = 14 treatments) were analyzed. The ranges for UN/NI, manure-N, and plasma $\Delta^{15}\text{N}$ were 0.28 to 0.65 g/g (average = 0.48 g/g; SD = 0.12), 19.34 to 75.20 g/day (average = 40.72 g/day; standard deviation (**SD**) = 19.20), and 3.41 to 5.70 ‰ (average = 4.85; SD = 0.74), respectively. The UN/NI was more strongly correlated ($r_s = 0.89$) with plasma $\Delta^{15}\text{N}$ than manure-N ($r_s = 0.69$). Linear regression analysis showed that the UN/NI had a positive correlation ($r^2 = 0.84$, $P < 0.001$) with plasma $\Delta^{15}\text{N}$. A weaker, but positive ($r^2 = 0.34$; $P < 0.05$) relationship between manure-N and plasma $\Delta^{15}\text{N}$ was also observed. In addition, REML estimates of the parameters in linear mixed-effects models illustrated that there were significant relationships between both UN/NI and manure-N with plasma $\Delta^{15}\text{N}$ ($P < 0.01$ and $P < 0.05$; respectively). These findings underscore the potential of plasma $\Delta^{15}\text{N}$ as a reliable, non-invasive tool for improving N feeding strategies in small ruminants, with implications for mitigating greenhouse gas emissions and enhancing environmental sustainability in livestock systems.

204 Carcass characteristics and meat quality of Nguni yearlings feedlotted with climate-resilient *Leucaena leucocephala* leaf meal

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Beef farmers commonly utilize agro-processing by-products like oil cake meals (**OCM**) of soybean, sunflower, and cotton as protein sources when formulating feedlot diets. Nonetheless, these OCM are costly and the high fat content of OCM impairs carcass characteristics and meat quality. Moreover, low fibre content restricts OCM usage in cattle fattening, particularly for indigenous breeds like Nguni that are well-known for flourishing on fibrous diets. Conversely, fattening cattle with climate-resilient fodder trees such as *Leucaena leucocephala* (*Leucaena*) has a potential to address this research gap. A study was conducted to evaluate the carcass characteristics and meat quality of Nguni yearlings feedlotted with *Leucaena* leaf meal. Experimental procedures were ethically approved by the ARC-Irene Animal Ethics Committee (APIEC18/11). *Leucaena* leaf meal was harvested from Hatfield Experimental Farm of the University of Pretoria, shade-dried for 3-5 days, milled (2 mm), and used to formulate experimental diets. *Leucaena* inclusion levels were 0%, 10%, 20% and 30%. Sixteen male Nguni yearlings (121.69 kg initial LBW) were randomly allocated to feedlot diets with 0%, 10%, 20%, and 30% *Leucaena* inclusion levels. On day 133 of feedlotting, animals (249.50 kg final LBW) were slaughtered, and carcasses were chilled at 2 °C for 24 hours before sampling. The *longissimus lumborum* (**LL**; 1st to 5th lumbar vertebrae), *semitendinosus* (**ST**; whole cut) and *biceps femoris* (**BF**; whole cut) were sampled. Carcass characteristics and meat quality traits were evaluated accordingly. Data was subjected to analysis of variance using SAS (2020) software. Means were separated by Shapiro-Wilk's test at 5% level of significance. Feeding animals with a 20% *Leucaena* diet resulted in higher total body weight gain of 151.75 kg compared to 123.00 kg for 0% *Leucaena* diet. Animals on a 20% diet had the highest eye muscle area (5340.70 mm²) over other treatment groups. Furthermore, 20% *Leucaena* inclusion improved meat tenderness with MFL of 16.37 µm over 19.08 µm for 0% *Leucaena* diet at day 14 of carcass aging. Meat pH of animals fattened with a 20% *Leucaena* diet was significantly lower (5.89) than that of animals fed with a 0% *Leucaena* diet (6.10) after 1 hour of chilling. Meat temperature of animals fed with a 20% *Leucaena* diet was significantly higher (29.58 °C) than that of animals fed with a 10% (26.10 °C) and 30% (25.20 °C) *Leucaena* diets after 3 hours of chilling. Additionally, all experimental animals had a meat pH range of 5.58-5.72 after 24 hours of chilling. This is within the acceptable range of 5.40-5.70, which is regarded as carcass from unstressed animals. Higher meat redness was observed in animals fed with a 20% *Leucaena* diet (9.27) over that of a 30% *Leucaena* diet (7.30). Nevertheless, absorbance of beef from Nguni yearling feedlotted with a 30% *Leucaena* diet was significantly higher (0.85) than that of Nguni yearlings fed with a 20% *Leucaena* diet (0.73) at 730 nm. A 20% *Leucaena* diet improved carcass characteristics and meat quality of Nguni yearlings. A further study is underway on fatty acids profiling and sensory evaluation of *Leucaena* fattened beef.

236 Nutritive value assesment of some agro-industrial by-products as an alternative strategy in ruminant feeding systems

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World's human population is in constant growth and supposed to reach 8.6 billion people in 2030 and 9.80 by 2050. Consequently, there is a need to increase food production. In Tunisia, more than the third part of produced vegetables are lost either during harvesting or processing of food for human consumption. The potential use of these by-products could reduce the environmental impact of this waste and favour directly the development of circular economy. The aim of this present study was to determine the nutritional value of some vegetable wastes (lettuce leaves, artichoke (stem/leaf), tomato pulp and carrot leaves) commonly used in small ruminant diets, particularly sheep in the region of Sidi Bouzid (Center of Tunisia). The samples bought from the local market, air dried, then ground and subjected to analysis for chemical composition, digestibility and kinetics of in vitro gas production. Tomato pulps revealed the highest organic matter (**OM**) content (94% DM), while lettuce leaves recorded the lowest levels (73% DM). The crude protein (**CP**) varies ($P < 0.001$) from 7.5% (artichoke stem) to 18% (tomato pulp and carrot leaf). A significant variation ($P < 0.001$) was also observed between the different wastes for their cell wall contents. Thus, lettuce leaves recorded the lowest concentration of NDF (22%) and ADF (16%). However, tomato pulps contained the highest NDF and ADF contents, being 56 and 43%, respectively. It is pertinent to mention that cell wall lignification varied widely between vegetable wastes ranging from 17% (carrot leaves) to 32% (lettuce leaves). Total phenol contents (**TP**) reached the highest levels ($P < 0.001$) in artichoke stems (139 mg EAG / g DM) while the lowest were detected with lettuce and carrot leaves (57 mg EAG / g MS). The other sub-products contained (artichoke leaves and tomato pulp) recorded intermediate levels (93 mg / gMS). Determination of the inhibitory power (IC50) of the different samples proved that lettuce and carrot leaves have the lowest antioxidant activity (**AA**).

Likewise, in vitro fermentation of substrats, based on the use of gas production tool pointed out significant variations ($P < 0.001$) between all the studied parameters of kinetics of gas production. The highest values ($P < 0.001$) of gas production at 24h, metabolizable energy (**ME**), organic matter digestibility MO (**dMO**) and total volatile fatty acid **VFA** were recorded in tomato pulps and carrot leaves. It was concluded that the vegetable wastes studied herein could be classified as an excellent source of nutrients potentially used in sheep feed. Some could be considered a local protein source, others as an energy source. The good management of these wastes could lead to both reducing animal production cost, environmental pollution and emission of greenhouse gases.

286 Methodological framework to evaluate circularity in livestock systems: application to different contexts in Europe

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The specialization of modern agricultural and livestock systems has increased land productivity to meet the demand for food but has led to a greater dependence on external inputs based on fossil fuels (fertilizers, concentrates). The spatial decoupling between the components of the agri-food system has led to significant imbalances that end up as emissions, generating impacts on a local and global scale. The transition towards circular food systems is therefore imperative, and livestock can play an important role in it by i) enabling use of biomass resources unsuitable for direct human consumption and ii) facilitate recycling of nutrients within and across systems. Jointly assessing circularity and environmental impact in livestock systems is challenging. While life cycle assessment (**LCA**) has been applied for this purpose, impact categories and/or functional units of LCAs do not always capture the actual implications for resource flows in agri-food systems. Specific approaches to assess circularity are needed. The purpose of this study is to i) propose a selection of LCA and circularity indicators to be applied in livestock systems within a consistent methodological framework and ii) discuss their strengths and challenges when tested on case studies applying circular strategies. Considering the context and goal of the project, a definition of circularity especially adapted for livestock production was proposed, aligned with circular economy principles. Previous research initiatives, guidelines, and scientific literature were gathered and reviewed. The applicability of different metrics and approaches identified to assess circularity in agri-food systems was explored, and a specific framework was developed to categorise the resource flows involved in livestock systems. As a result, a selection of indicators and methods for assessing the circularity of livestock systems was proposed. Metrics were categorized according to the aspect of circularity (circular strategy) that they can measure: 1) minimize resource use, 2) increase recovery and 3) reduce losses. Finally, their applicability was tested on a selection of six case studies, involving circularity actions in different contexts: 1) use of bakery by-products for animal feeding (Italy), 2) sowing legume-rich species in Mediterranean pastures (Spain), 3) seasonal grazing in alpine pastures (Italy), 4) solid-liquid separation of slurry (UK), 5) anaerobic digestion of manure (Germany), and 6) use of grass-clover swards (Norway). Actions based on increasing recovery of nutrients or reducing their losses, produced positive effects on circularity, captured through indicators like Nutrient Use Efficiency, Nutrient Recycling Index, and Circularity of Outputs. These strategies also resulted in reductions of greenhouse gas (**GHG**) emissions. Circularity actions focusing on minimizing resource use showed improvements in metrics like edible Protein Conversion Ratio, Land Use Ratio and Circularity of inputs, but their effect on GHG emissions produced contrasting or limited results. This highlights the importance of selecting appropriate metrics and system boundaries according to different situations, as well as the need to carefully consider trade-offs when assessing strategies for jointly promoting circularity and climate mitigation in livestock systems.

324 Mitigating greenhouse gas emissions in tropical agriculture: the impact of crop-livestock integration practices in Guadeloupe

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Tropical agricultural systems are particularly vulnerable to climate change. As the agricultural sector is a major contributor for greenhouse gas (**GHG**) emissions, it is necessary to identify agroecological practices that may reduce GHG emissions. The low synthetic input requirements of agroecological practices make them *a priori* less GHG emitting than conventional agriculture practices and can even act as GHG sinks. This study aimed to evaluate the mitigation potential of crop-livestock integration (**CLI**) in tropical agroecosystems.

We hypothesized that a high degree of CLI leads to lower GHG emissions. Data collection was conducted in a tropical environment, on 6 Guadeloupean mixed crop-livestock farming systems (System 1 to System 6, S1-S6). The ecological network analysis (**ENA**) methodology was used to characterize farming systems structure and management practices, according to nitrogen flow dynamics, especially nitrogen flows between compartments (of livestock, crops and storage). Moreover, ENA was used to estimate indicators of productivity, dependency, resilience and efficiency. Based on this input-output model, the GHG balance over a one-year period was estimated using IPCC 2019 guidelines to compare systems' total methane (**CH₄**) emissions (enteric and manure) expressed in g/day or g/per Tropical Livestock unit cattle (**TLU**, which is equivalent to a cow weighing 250kg live weight). S1 was the less complex system, with 2 TLU, a crop production (coconut copra and water) and a composting unit. In contrast, S6 is composed of 76.3 TLU (cattle, *n*= 3; goat, *n*= 62; poultry, *n*= 6000) and food crop production (lettuce). Preliminary results indicate that the most integrated system (S1) showed lower total CH₄ (enteric and manure) emissions as compared to the least integrated system (S6). Preliminary results indicate that enteric CH₄ emissions represent more than 90% and 66% of total CH₄ emissions, for system S1 and S6, respectively. The CH₄ emissions derived from manure storage represent less than 1% and 33% of total CH₄ (enteric and manure storage) emissions (g/day or g/TLU) for system S1 and S6, respectively. This study needs further development with larger farm samples and may provide indication on practices in tropical regions that reduce GHG emissions and promote nutrient recycling (nitrogen and/or carbon) within the system.

340 Harnessing source-separated organic municipal waste for fertilizer production and sustainable waste management in Ethiopia

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This study addresses the challenge of inefficient organic municipal solid waste (**MSW**) management in rapidly urbanizing areas like Ethiopia, where poor waste handling practices contribute to environmental pollution, public health risks, and significant greenhouse gas (**GHG**) emissions, particularly methane. The study introduces an innovative approach to enhancing composting efficiency for organic MSW and improving fertilizer quality using a pilot Aerated Static Pile (**ASP**) system combined with extremophilic lake sediment as a microbial inoculum. By optimizing waste mixing ratios, fine-tuning operational parameters, and evaluating the quality of the resulting organic fertilizer, this research offers valuable insights into sustainable waste management solutions tailored to fast-growing urban areas. The study investigated different combinations of biodegradable MSW specifically food waste and yard waste along with cow dung and extremophilic lake sediment used as microbial inocula. Key composting parameters, including pH, carbon-to-nitrogen (**C/N**) ratio, moisture content (**MC**), and electrical conductivity (**EC**), were systematically monitored throughout the composting process to identify optimal operating conditions. Additionally, heavy metal concentrations were measured before and after composting to evaluate the safety and regulatory compliance of the final compost product. Results revealed optimal conditions with pH values between 5.43 and 7.89, a C/N ratio ranging from 23:1 to 42:1, MC levels between 60% and 66%, and EC values from 4.10 to 5.24 mS/cm. Heavy metal concentrations consistently met safety requirements. Extremophilic lake sediment (Run I) outperformed cow dung (Run II) as a microbial inoculum, with Pile P3 (50% food waste, 50% yard waste) achieving the highest-quality compost, followed by Pile P2 (75% food waste, 25% yard waste). Conversely, Pile P4 (25% food waste, 75% yard waste) experienced lower temperatures, resulting in slower decomposition rates. The study highlights that extremophilic lake sediment enhances decomposition efficiency and produces superior compost quality compared to conventional inocula like cow dung. Importantly, the research is framed within the principles of a circular economy, promoting resource recovery, waste-to-value innovation, and nutrient cycling. The ASP method with lake sediment inoculum provides a cost-effective solution by reducing reliance on chemical fertilizers and decreasing landfill burdens. Preliminary cost assessments suggest that the use of ASP technology can lower operational costs over time due to faster composting cycles and reduced environmental remediation expenses. Moreover, initial stakeholder engagement and user surveys indicate a strong willingness among urban farmers and local waste cooperatives to adopt the technology, particularly when compost quality is high and economic returns through improved crop yields and soil health are evident. In conclusion, this study demonstrates that ASP composting with extremophilic lake sediment can play a vital role in advancing circular economy strategies by reducing organic waste, cutting GHG emissions, and producing safe, high-quality compost. The approach not only enhances ecological sustainability but also presents a scalable and economically viable model for sustainable waste management and climate change mitigation in Ethiopia and similar rapidly urbanizing regions globally.

382 Assessing nutrient circularity on different farm typologies in South Africa

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Livestock production contributes approximately 25% of Africa's agricultural gross domestic product (**GDP**), making it a vital component of rural livelihoods. Cattle manure is an underutilized resource in this context, offering significant potential for improving soil fertility, pasture productivity, and overall system sustainability. Enhancing nutrient circularity within and between production systems is key to promoting resource efficiency, profitability, and environmental resilience.

This study aimed to assess the current implementation of nutrient circularity practices across different agro-ecological zones and cattle farm typologies in the Limpopo, Free State, and North West provinces of South Africa. Data was collected through structured questionnaires and in-person interviews with both small-scale and commercial cattle farmers.

Preliminary findings reveal a significant disparity in manure management practices. Only 10% of small-scale farmers actively recycle manure, indicating a lack of resources, awareness, or access to appropriate technologies. Among commercial farmers, 35% reported using compost made from manure, bedding, and wasted feed demonstrating partial adoption of nutrient recycling strategies.

Interestingly, while high-density grazing (**HDG**) systems have been shown to offer benefits such as improved pasture quality, enhanced reproductive performance, and reduced reliance on chemical tick control, 90% of commercial beef farms surveyed did not implement HDG. Follow-up discussions suggest several barriers to adoption, including limited knowledge of HDG principles, perceived labor intensiveness, infrastructure constraints (such as fencing), and uncertainty around short-term economic returns. These findings underscore the importance of targeted extension services and policy incentives to support the transition to more circular and regenerative livestock systems.

In conclusion, the results highlight both the potential and the current gaps in implementing nutrient circularity on South African cattle farms. Ongoing research and engagement with farmers will further inform strategies to scale up sustainable manure and grazing management practices in diverse farming contexts.

405 Assessing the system stability and performance of small-scale biogas digesters across seasons

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Growing demand for sustainable energy and fertilizer-drives adoption of manure-based biogas-digesters, especially in smallholder-systems. However, lack of insulation and seasonal-feed variations reduce digester stability and performance across-seasons. The objectives of this-study were, to: (1) analyse seasonal-variations in the composition and chemistry of cattle manure for typical semi-intensive smallholder-cattle, (2) assess the impact of these variations along with seasonal temperature changes, on digester stability and performance, as well as the quality of the biogas and digestate. The experiment was conducted using a 6 m³ prefabricated-digester, which was monitored for biogas production performance and process-stability from May to November 2023 in Nairobi, Kenya. The study analysed key parameters such as daily biogas production, composition, digester and ambient temperature, and physico-chemical properties of manure and digestate. Additional *in vitro* batch test was conducted to determine the maximum potential methane-production from manure at controlled temperature of 37°C for 55 days of hydraulic retention times (HRT). Pearson correlation coefficients (r) were calculated to assess the linear relationship between key physico-chemical parameters (e.g., carbon to nitrogen (C:N) ratio, total-solid (TS), volatile-solid (VS)), temperature (ambient and digester), and biogas production. Statistical significance was assessed at $p < 0.05$. The results revealed significant seasonal variations in the physico-chemical composition of cattle manure, with a C/N ratio ranging between 18.12 and 33.49 ($p = 0.011$), phosphorous 2.16–5.27 g/kg ($p = 0.042$), carbohydrate 16.34–25.70% ($p = 0.005$), hemicellulose 18.74–25.40%, ($p = 0.023$), TS 7.27–9.02% ($p = 0.011$), and VS 5.74–7.46% ($p = 0.012$). During this study-period, ambient temperature in Nairobi varied significantly ($p = 0.001$), with the coolest months being July and August average of 16.9°C, and the warmest months occurring between October and November average of 19.7°C. The digester temperature closely mirrored air temperature patterns, with the lowest monthly average in July and August (18.3°C) and the highest in October and November (21.5°C). Daily air temperature fluctuations ranged from 9.9°C to 29.3°C in August and 12.6°C to 30.2°C in November, while daily digester temperature fluctuations ranged from 13.9°C to 25.6°C in August and 13.2°C to 29.6°C in November. The highest biogas (871.2 mL/g VS) and methane (499.5 mL/g VS) yields were recorded in October, while the lowest biogas (310.3 mL/g VS) and methane (172.6 mL/g VS) yields occurred in July. So that digester-temperature ($r = 0.957$, $p = 0.003$), ambient-temperature ($r = 0.862$, $p = 0.027$), and C:N ratio ($r = 0.953$, $p = 0.003$) exhibited the strongest statistically significant correlations with biogas production. A clear relationship was established between temperature variations in the digester, ambient air and biogas performance. TS and VS conversion-rates were 25.85% and 33.12%, respectively, in the household digester, compared to 74.01% and 77.70% in the temperature-controlled (37°C) and long HRT (55 days) *in-vitro* experiment. This shows limited farm-scale digester efficiency due to short HRT and temperature fluctuations, highlighting optimization needs in feeding, HRT, outlet design, and temperature control.

435 Cactus *Opuntia stricta* contributes to carbon sequestration and modulates soil properties in Laikipia, Kenya

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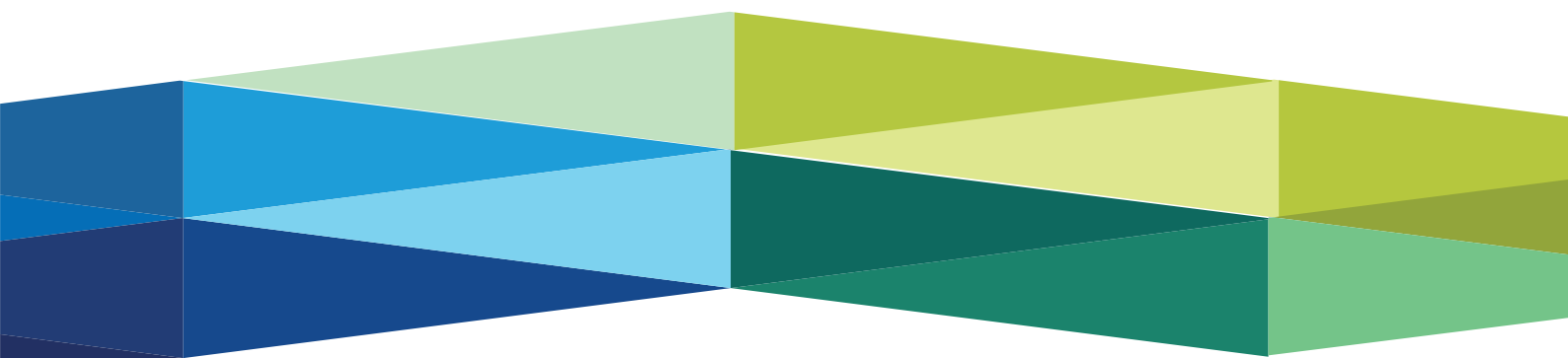
Soil degradation and climate change are pressing concerns in arid and semi-arid regions, such as Laikipia, Kenya. The invasive cactus *Opuntia stricta* has been reported as a threat to Kenya's pastoral livelihoods. However, the current management strategies largely focus on eradication, often overlooking their potential contributions to ecosystem services. This is attributed to the lack of data on ecosystem services provided by *O. stricta*, which could support decisions on either sustainable management or replacement with non-invasive, spineless varieties. This study evaluated the effects of *O. stricta* on carbon sequestration and its influence on soil properties. A randomized complete block design with ten replications, each measuring 30 x 30 meters, was used. Treatments were soil sampled at a depth of 20 cm from beneath and outside the clusters of cacti. For each treatment, soil samples were collected from five random points within each block and composited for analysis. The above and belowground biomass were also evaluated. Significant differences were observed within and outside the clusters for pH 7.5 and 7.1, organic carbon 0.9 and 0.7%, organic matter 1.5 and 1.2%, calcium 2112.4 and 1850.0 Meq/100kg, potassium 1023.0 and 385.1 Meq/100kg, Manganese 114.1 and 62.8 mg/kg respectively. The aboveground biomass ranged between 1.1 to 4.2 t ha⁻¹ DM translating to between 0.5 to 1.9 t ha⁻¹ of carbon equivalent. Similarly, the root biomass at the center of clusters ranged between 4.6 to 14.0 t ha⁻¹ DM. These findings underscore the role of *O. stricta* in enhancing soil properties and its potential to sequester carbon, offering insights for sustainable management strategies in Laikipia and similar regions.



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Conference

Theme 7: Scaling and modelling of greenhouse gas emissions



18 Greenhouse Gas Emissions from Livestock Watering Points Along Tropical Streams in Taita Hills, Kenya

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Livestock keeping is seen as the solution to food insecurity in Africa but contributes significantly to greenhouse gas (CO_2 , CH_4 , N_2O) emissions in agropastoral and pastoral landscapes. To determine the influence livestock production on greenhouse gas emissions from stream and rivers, a four-sampling campaign transitioning from wet to dry season (early dry, late dry, early wet and late wet season) was conducted along livestock density gradient in the Afromontane-savanna in Wundanyi and Bura Rivers in Taita-Taveta County, South-eastern Kenya. Sampling sites were categorized into reference sites (REF, 7 in number), low density livestock sites (LDL, 4), and high-density livestock sites (HLD, 4). In the HLD sites, there were high levels of dissolved organic carbon (DOC mg/L), fine benthic organic matter (FBOM, expressed as a percentage), temperature ($^{\circ}\text{C}$), and particulate organic matter (POM mg/L), as well as ammonium (NH_4 mg/L) compared to LLD and REF sites. In contrast, the levels of dissolved oxygen (DO, measured in mg/L), nitrates (NO_3 mg/L), and nitrites (NO_2 mg/L) were lower than REF and LLD sites. There were also high CH_4 and CO_2 fluxes in HDL sites compared to LLD and REF sites while N_2O flux decreased with increased in livestock density. Based on seasonality, high levels of DOC, POM, FBOM, CH_4 flux, CO_2 flux and low DO levels were recorded in dry season compared to wet season. There was positive relationship of FBOM%, DOC, NH_4 , and NO_3 with CO_2 and CH_4 fluxes. This suggests that respiration and methanogenesis were responsible for CO_2 and CH_4 emissions, respectively. The positive association of N_2O with NH_4 , NO_2 and NO_3 , and the negative relationship with DOC and FBOM% suggested that nitrification may have controlled N_2O production. In addition, negative fluxes of N_2O occurred in HLD and LLD sites suggested complete denitrification of N_2O to N_2 . From the results, livestock density is a major contributor of CH_4 and CO_2 fluxes in Afromontane-savanna rivers. Hence, increased livestock densities accessing watering points along rivers will likely increase the African continent's greenhouse gas budget. From the results, livestock density is a major contributor of CH_4 and CO_2 fluxes in Afromontane-savanna rivers. Hence, increased livestock densities accessing watering points along rivers will likely increase the African continent's greenhouse gas budget.

31 Modelling GHG emissions by Kenya's agricultural sector for forecasting future values: comparative analysis of ARIMA, ETS and NNAR models

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In order to calculate greenhouse gas (GHG) emissions in a country, emission of each GHG is converted into megatons of carbon dioxide equivalent based on its potential to cause global warming. Modelling GHG emissions for predicting future values continues to be highly challenging to many environment researchers. Autoregressive integrated moving averages (ARIMA) models are the simplest time series models used. Other models used are error, trend and seasonality (ETS) models not requiring data to be stationary as is the case for ARIMA models and neural networks autoregressive (NNAR) models that apply neural networks in the analysis of time series data.

The aim of the present research study was to identify whether NNAR models achieve more or less accurate forecasts when compared to ARIMA and ETS models, when modelling GHG emissions from agricultural activities (both growing of crops and rearing of animals) in Kenya to forecast future values.

The data analyzed for the study was on GHG emissions by agricultural sector in Kenya for the period starting from 1970 to 2023 sourced from the International Monetary Fund (IMF) database. ARIMA, ETS and NNAR models were investigated. The best specification for the models was identified automatically using a step-wise procedure implemented using corrected or uncorrected Akaike Information Criterion. The best ARIMA, ETS and NNAR models were assessed for independent residuals using Ljung-Box test and residuals having a constant variance using the ARCH LM test. These models were then compared in terms of their in-sample and out-sample forecast accuracy as indicated by root mean squared error (RMSE), mean absolute error (MAE), mean absolute percentage error (MAPE) and mean absolute scaled error (MASE). Diebold-Mariano (DM) test was used to identify whether difference in forecast accuracy between models was statistically significant. Validation of the models was undertaken using train-test split method undertaken using the 80:20 rule.

Best model was ARIMA (0, 1, 0) model for the AIMA models, ETS (M, A, N) model for the ETS models and NNAR (1, 1) for the NNAR models. Out-sample RMSE was highest for ARIMA (0, 1, 0) model (RMSE = 0.088) and lowest for the NNAR (1, 1) model (RMSE = 0.334). MAE, MAPE and MASE were highest for ETS (M, A, N) model (MAE = 0.073, MAPE = 1.816, MASE = 1.339) and lowest for NNAR (1, 1) model (MAE = 0.299, MAPE = 7.242, MASE = 5.488). Out-sample forecast accuracy was significantly lower for NNAR (1, 1) model than for ETS (M, A, N) model (DM = 3.787, $p = 0.004$) and ARIMA (0, 1, 0) model (DM = 3.620, $p = 0.005$), but was not significantly different between ETS (M, A, N) and ARIMA (0, 1, 0) models (DM = -0.607, $p = 0.557$).

The study, therefore, recommended that NNAR models should not be preferred instead of ARIMA or ETS models when modelling GHG emissions by agricultural activities of a developing countries in Africa such as Kenya for forecasting future values.

32 Interdisciplinary benefits and trade-offs in carbon reduction practices for sheep farming

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Land managers are challenged with balancing multiple concurrent priorities, including adaptation to the changing climate, reducing or removing greenhouse gas (GHG) emissions, conserving biodiversity habitat and improving enterprise profit. As such, the demand for innovations that will enhance productivity, profitability and biodiversity while reducing GHG emissions has never been greater. We conducted people-centric design with seven sheep farmers across Australia to ascertain biophysical, economic and biodiversity effects of 13 interventions. Baseline systems were refined over several cycles with case study farms to ensure model output rigour. Systems interventions were then imposed on calibrated baseline systems. Interventions were aimed at reducing GHG emissions while maintaining profitability and improving biodiversity, including planting native tree species, renovating pastures with anti-methanogenic species, improving liveweight gain via grazing management, adopting low-emissions feed additives and animal genetic improvements (lower methane yield and/or wool growth). Demand-driven interventions informed by producers tended to improve profit and production but also increased GHG emissions due to positive coupling between productivity and GHG emissions. Standardised interventions imposed on all farms (such as planting 10% of grazing area with trees) had the greatest benefit for net GHG and emissions intensity, more so than low-emissions feed supplements seaweed (*Asparagopsis taxiformis*) or 3-nitrooxypropanol (3-NOP). Planting trees was the only intervention that improved biodiversity while reducing net GHG emissions (-120 to -15%), but generally decreased gross margins (-56 to 22%). Introducing genotypes with ultrafine wool generated the greatest economic benefit. Nature-based solutions were more economical than carbon reductions, particularly interventions aimed at reducing enteric methane, and more conducive to biodiversity preservation. Despite this, carbon sequestration in soils and vegetation diminished over time and were reversible, whereas carbon removals were more consistent each year, permanent and cumulative. We found no evidence of an innovation panacea that reduced or removed GHG while concurrently improving production, profit and biodiversity. We conclude that the greatest benefit emanates from those interventions that addresses the underperforming areas, be it economic, environmental, psychological, institutional or otherwise. In this vein, contextualised bundling of innovations may be more prospective than singular interventions, particularly when those interventions target suboptimal performance in biodiversity, productivity, profitability, GHGs, or combinations thereto.

48 Evaluating impact of climate change on soil organic carbon stock in Anjeni Watershed, North-West Ethiopia

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Assessing soil organic carbon (SOC) stock is essential to minimizing the consequences of climate change (CC) and enhance sustainable agriculture since CC has a substantial impact on SOC decomposition and, eventually, carbon dioxide emissions from the soil into the atmosphere. Therefore, the objective of this study was to employ the RothC model to simulate SOC stock in the Anjeni watershed under climate change with varying land use and slope gradient. Following validation of the RothC model using long-term observed climate data and inputs from soil and land management, SOC stocks was simulated for 2052 using the most recent climate scenarios known as "Shared Socioeconomic Pathways" (SSP) SSP5-2.6 and SSP5-8.5. The findings indicated that all scenarios predicted temperature increases, with the lowest rise of 0.24 °C based on HadGEM3 (SSP5-2.6) in January and the maximum rise of 5.06 °C based on MPI (SSP5-8.5) in September. The major rainy season rainfall is projected to increase by 8.1-16.5% by 2052, while the dry and minor rainy season rainfall was inconsistent. The projected SOC increased in grass/fallow land from 1.04 t/ha (GFDL_SSP5-2.6) to 2.2 t/ha (IPSL_SSP5-8.5) at the lower slope gradient. The highest increase in SOC was predicted by MPI (SSP5-2.6 and SSP5-8.5). The SOC at the lower slope gradient grass/fallow land was anticipated to increase from 1.04 ton/ha (GFDL_SSP5-2.6) to 2.2 ton/ha (IPSL_SSP5-8.5), as was the case with almost all of the GCM scenarios. MPI showed the most rise in SOC (SSP5-2.6 and SSP5-8.5). Overall, the SOC stock in the Anjeni watershed simulated to rise under all climate scenarios, which could boost agricultural productivity and reduce emission. The current soil-water conservation initiatives must be supported in order to maintain the reference SOC stock, and promote climate-smart agricultural practices especially in upper slope cultivated lands that further increase the SOC for sustainable agriculture.

52 Carbon Footprint of dairy and suckler beef in Denmark: baseline and potential mitigation alternatives

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The aim of this study is to assess the climate change (**CC**) impacts of Danish beef production, with a relatively granular model that accounts for different production systems (conventional and organic), breed types, and animal groups. Potential strategies (12 scenarios) aiming to reduce CC impacts are also investigated. The baseline year of the assessment is 2021.

The average age and the carcass weight (**CW**) at slaughter of the different animal groups were defined based on the Danish cattle database ("Kvægdatabasen"). Each animal group was associated with average housing, manure, and grazing conditions (defined based on actual farmer data, available via "MarkOnline"), and typical feed rations (calculated via DMS_NorFor), during grazing and housing periods. Enteric fermentation emissions were calculated based on the IPCC (2019) Tier 2 approach reported in the Danish National Inventories. Methane and nitrous oxide emissions related to the excreted manure were calculated as a function of the housing type and grazing time, based on standard national values and IPCC (2019) emission factors. The upstream CC impacts related to the production of feed were mostly retrieved from Agri-footprint 6.3. The environmental impacts of dairy cows were distributed between the sold milk and the liveweights of cows and calves following the IDF 520/2022 guideline (i.e., a biophysical allocation (milk//liveweight), followed by mass allocation (liveweights of cows//calves)), whereas the ones of suckler cows were only distributed between the liveweights of cows and calves (mass allocation).

In 2021, Danish slaughtered cattle contributed to 8.10×10^9 kg CO₂eq, where 2.21×10^9 kg CO₂eq was attributed to the produced beef (CW), while the rest was attributed to the produced milk. The average CC impact of Danish beef in 2021 was 17.9 kg CO₂eq / kg CW (16.1 and 26.3 kg CO₂eq / kg CW for dairy beef and suckler beef, respectively). In 2021, dairy beef represented 82% of the beef produced in Denmark.

Scenario analyses showed that the potential use of 3-NOP additives in dairy cow's feed ration can have a mitigating effect on the CC impact of beef: from 17.9 to 17.4 kg CO₂eq /kg CW (3.1% reduction), and from 2.21×10^9 to 2.14×10^9 kg CO₂eq when focusing on the national impacts attributable to the produced beef (CW). Combining the 3-NOP scenario with potential changes in the Danish beef production looking towards a reduction of the suckler cow population and an increase in the dairy population, achieved by avoiding the current export of 1 months old calves (keeping the total meat produced constant), showed a potential reduction in the average CC impacts of beef from 17.9 to 16.5 kg CO₂eq /kg CW (8.1% reduction) – a comparable reduction potential was also evaluated for the national impacts attributable to the produced beef (CW).

The average CC impact of Danish beef in 2021 was 17.9 kg CO₂eq / kg CW. Noteworthy reductions in the beef CC impacts may be achieved by using 3-NOP in the cow's feed rations, and by reducing the population of suckler cattle in favor of more dairy cattle.

56 Reducing the carbon footprint of Québec Dairy Farms: A collaborative Living Lab approach

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Milk production contributes to greenhouse gas (GHG) emissions, primarily through methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2). To address this, the Dairy Farmers of Canada have set a target of achieving carbon neutrality by 2050. In line with this vision, 20 dairy farms from the province of Québec in Canada are participating in a Living-Lab initiative. The aim of this project is to assess carbon footprint and understand the selection by farmers of innovative strategies to lower GHG emissions while enhancing carbon sequestration through a living lab. The farms are located in four distinct regions of the province and encompass a wide variety of farming models. As a start, we have established a baseline carbon assessment using 2022 as the reference year. Producers provided data, including information on herd management, animal feeding, field practices, manure and bedding management, energy use, and technical-economic aspects of farm operations. A visit was conducted to each farm to gain a deeper understanding of their practices and context. Four carbon assessment calculators (Holos, Cool Farm Tool, Logiag, and Agriclimat) were used to evaluate the carbon footprint of each farm with a comparison between methodologies, highlighting any discrepancies and their implications. The results, from the Holos calculator, showed that the average carbon footprint of milk across the 20 farms was of 0.96 ± 0.19 kg CO_2 eq. per kilogram of fat- and protein-corrected milk (**FPCM**; mean $\pm$ standard deviation (**SD**)). It ranged from 0.69 to 1.55 kg CO_2 eq. per kg of FPCM. On average ($\pm$ SD), animals CH_4 contributed to $60.0 \pm 20.0\%$ of total emissions allocated to milk production, followed by manure CH_4 and N_2O at $27.5 \pm 12.3\%$, fields N_2O at $15.8 \pm 8.3\%$, and inputs CO_2 eq. at $4.2 \pm 1.8\%$. Additionally, the soils of the farms sequestered an average of $7.5 \pm 6.3\%$ of their carbon emissions. Based on the interpretation of the initial carbon assessment and the farm specific practices, tailored best management practices (**BMPs**) have been identified in collaboration with researchers and producers. Some farms selected diet modifications to lower enteric fermentation, while others will adopt practices to mitigate emissions from manure storage, through storage duration, covering and additives. Even if enteric methane was the largest source of GHG emissions on farms, the majority of the farms have selected practices linked to the fields aimed to reduce GHG emissions and enhance carbon sequestration. The easiness of implementation and perceived potential impact on farm profit were also drivers of the decisions. This initiative demonstrates a practical, science-driven approach to integrating carbon footprint reduction strategies within the dairy sector, contributing to Canada's broader climate goals, while staying in touch with producer reality, interest and limitations.

57 Evaluation of models of enteric methane emissions in finishing steers

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Adequate estimation of enteric methane (**CH₄**) emissions in the ruminant sector is necessary for properly determining GHG emissions and developing measuring, reporting, and verification programs. However, measuring enteric CH₄ emissions under commercial conditions presents challenges due to technical and economic constraints. Thus, using prediction models allows for estimating individual enteric CH₄ emissions according to animal and dietary characteristics. When evaluated in independent datasets, there is limited information regarding the accuracy and precision of the reported equations to predict enteric CH₄ emissions in growing steers consuming a finishing diet. This study evaluated the predictive performance of various reported models for estimating enteric CH₄ production in finishing steers.

Data used to assess the prediction equations came from 446 growing steers from five experiments during the finishing phase. Diets were based on steam-flaked corn (ranging between 60 to 65% on a dry matter basis) and corn silage (ranging between 20 to 27% on a dry matter basis). Gas flux, nutrient consumption, and animal growth performance were individually evaluated. Pearson correlation was used to determine the association between enteric CH₄ production and the evaluated variables. In addition, seventy-two models were compared based on the mean square prediction error, the decomposition of the root mean square prediction error, and the concordance correlation coefficient (**CCC**).

Intake of dry matter ranged between 7.86 and 15.89 kg/d, representing an average of 2.0 kg of dry matter per 100 kg of body weight. Enteric CH₄ emissions varied between 113.4 and 299.8 g daily. The production of CH₄ was positive and significantly ($P < 0.05$) associated with body weight (0.34), average daily gain (0.17), and dry matter intake (0.18), resulting in a positive relationship with crude protein, neutral detergent fiber, hemicellulose, non-structural carbohydrates, starch, ash, and energy intake. However, intake of cellulose, acid detergent fiber, lignin, and ether extract did not show a significant association ($P > 0.05$) with CH₄ emissions. In addition, the forage proportion in the diet was negative and significantly ($P < 0.05$) associated with enteric CH₄ emissions (-0.27). Prediction models for estimating enteric CH₄ emissions showed lower sensitivity with root mean square prediction error (as a percentage of the observed mean) ranging from 17.79 to 99.21 and CCC ranging from -0.07 to 0.21. The decomposition of the root mean square prediction error showed mean bias (as a percentage of the root mean square prediction error) ranging from 0.14 to 94.87 and slope bias (as a percentage of the root mean square prediction error) ranging from 0 to 25.24. In addition, 49 models underpredicted, while 23 overpredicted enteric CH₄ emissions. The prediction of enteric CH₄ production showed a greater concordance correlation coefficient and lower mean square prediction error when the intake of dry matter and ether extract were included as predictors. These results suggest a limited ability to predict enteric CH₄ by growing steers during the finishing phase. Further efforts are required to generate sensitive models to accurately predict enteric CH₄ emissions in finishing steers.

80 Assessing the effect of conserved forage on dairy production and methane emissions in climate-resilient systems

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Dairy farming in both Victoria, Australia, and many parts of Africa relies on supplementary conserved forages, such as silage, hay, and straw, to meet feed requirements during periods when fresh pasture is unavailable, especially in regions with seasonal droughts or variable rainfall. Winter cereals, including barley, oats, and wheat, have become key forage sources due to their higher water-use efficiency and ability to thrive in dryland conditions. While much research focuses on their grain production potential, limited understanding exists regarding how different qualities of conserved cereal forages impact enteric methane (**CH₄**) emissions from dairy cows. Given that CH₄ is a major greenhouse gas (**GHG**) contributor on dairy farms, understanding its relationship with forage quality is crucial for improving the sustainability of dairy systems. This study investigates the effects of feeding high- and low-quality conserved forage on enteric CH₄ emissions and emission intensity in lactating dairy cows. The study was conducted on two dairy farms in northern Victoria, representing distinct farming systems. Baseline data for these farms were sourced from Munidasa et al. (2023). Metabolizable energy (**ME**) values for high- and low-quality hay and silage were obtained from the "Murray Dairy Fodder for the Future" project, which collected data across six locations in Victoria over two seasons. These ME values replaced baseline data, creating different feeding scenarios. The model incorporated animal dry matter intake (**DMI**), liveweight, and milk production to estimate CH₄ emissions. Standard deviations were used to assess the uncertainty in emissions and emission intensity. Statistical modelling was employed to compare the effects of high- versus low-quality forage on both absolute CH₄ emissions (kg CO₂e/head.day) and emission intensity (CH₄ emissions per unit of milk produced). Error bars representing the 95% confidence interval were used to quantify variability in the results. Enteric CH₄ emissions were found to be the largest contributor to on-farm GHG emissions. While changes in forage quality (high ME vs. low ME) led to some variation in absolute emissions, the effects on emission intensity were less significant. Farm A, which used a more intensive feeding system, had higher absolute emissions but lower emission intensity compared to Farm B, a more pasture-based system. Hay accounted for approximately 40% of the diet in Farm B and only 6% in Farm A, explaining why Farm A showed less variation in emission intensity across different forage quality scenarios. Despite higher emissions, Farm A, with greater milk production, resulted in lower emissions per unit of milk. These results suggest that while conserved forage quality can influence enteric CH₄ emissions, its impact on emission intensity is minimal when farm system characteristics such as feeding intensity and milk production are considered. Intensive dairy systems with higher milk yields may be more efficient in reducing CH₄ emissions per unit of product, even if total emissions are higher. Therefore, improving forage quality alone may not significantly mitigate GHG emissions. Rather, integrated farm management practices aimed at improving productivity and efficiency are essential for achieving more sustainable dairy systems with lower emissions.

137 Effect of temperature-humidity index on milk yield and methane emission in South African Holstein cows

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Negative impacts of heat stress in dairy cattle are expected to be aggravated by rising temperatures and increased incidences of heat waves, due to climate change and global warming. This postures a serious concern to animal productivity, including milk production, and consequently methane emissions. The Temperature-humidity index (THI) is a common index which is used as a proxy to evaluate heat stress in dairy cows in the sub-Saharan African climate (as reported in Livestock Science of 2020). However, information on the quantification of the effect of heat stress on milk production and methane emissions from dairy cattle under the subtropical environments of South Africa is still limited. This study, therefore, will assess the effects of heat stress as measured by THI on daily milk yield and methane intensity (kg methane / kg milk produced) in Holstein cows. Climatic weather data obtained from South African Weather Services for the Irene location was analysed together with 10,654 daily milk yield (kg/day) records of 65 Holstein cows, in their first to sixth lactations. This data was collected between 2023 and 2024, from the Agricultural Research Council's dairy herd at Irene near Pretoria in the Gauteng province. Temperature-humidity index was computed from daily maximal temperatures (°C) and minimum humidity (%). Methane intensity will be calculated from the average estimate of 354 grams of methane production per day and kg milk yield. Daily milk yield was analysed by generalised linear model procedure of the Statistical Analytical System, with THI treated as a categorical variable grouped into 5 classes (1= [60 - 64], 2= [65 - 68], 3= [69 - 72], 4= [73 - 76], 5= [77 - 80]), to evaluate the response of milk yield to THI levels. The preliminary results showed that THI significantly affected ($p < 0.0001$) daily milk yield. Milk yield increased from 21.77 ± 0.13 to 22.60 ± 0.12 kg/day when the THI increased from 65 to 72, but declined by 0.70 kg/day when the THI further increased from 72 to 80. A complete study will account for parity, lactation stage, cow age at calving and test-date effects on milk yield and methane emissions. A Greenfeed system has also been acquired in March 2025, and is used to measure actual methane production as part of the current study. This study will highlight the needs for precautionary measures, as mitigation strategies, against the loss of milk yield and increased methane emissions due to heat stress in dairy cows. It is confirmed that the results will be available before the conference and reported as such at the conference.

147 The farm-gate methane footprint of a weaner versus an ox-production system in diverse beef cattle

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This study aimed to quantify and compare the farm-gate methane footprint of a weaner versus an ox-production system for some of the major and diverse South African beef cattle breeds. The methane output (measured as methane intensity, kg methane/kg live weight) of a weaner calf production system for nine diverse beef cattle genotypes was simulated in a previous study (presented at the 8th GGAA conference). For this study, the results from the ox-production system are estimated and compared with the weaner production system. Based on data availability and relative numbers, the genotypes selected were the Afrikaner, Nguni, Bonsmara, Angus, Hereford, Brahman, Brangus, Charolais, and Simmentaler breeds. A farm size of 1 200 ha with a carrying capacity of 6 ha/Large Stock Unit was simulated, which could carry 200 Large Stock Units. Frame size-specific equations were used to estimate Large Stock Units for the different breeds. In South Africa, the enteric methane emission of a Large Stock Unit is estimated to be 94 kg methane/year (Tier II estimate). The methane intensity (kg methane/kg live weight) was calculated by dividing the annual methane emissions from the farm per year (200 Large Stock Units x 94 kg methane = 18 800 kg methane) by the total kg live weight that leaves the farm from (1) a weaner calf production system or (2) an ox-production system. The total live weight available for disposal annually was calculated by adding the total kg of surplus calves/oxen to be sold and the total kg of culled cows. In the case of the weaner production system, there was a large difference of up to 44% between the lowest and highest breed values. In the case of the ox production system, the differences were also enormous at 41%. On average the ox production system had a 18% lower methane intensity (0.55 versus 0.65). This can be explained by the difference in herd composition, e.g. in an ox-production system, much less productive cows are kept. Furthermore, breeds tend to rank differently, and the correlation in methane intensity between the two systems was only 0.32. The methane intensity values for the different breeds are (weaner production system value is the first) Afrikaner (0.60/0.57), Nguni (0.68/0.65), Bonsmara (0.59/0.52), Bonsmara (0.59/0.52), Angus (0.59/0.50), Hereford (0.64/0.52), Brahman (0.61/0.49), Brangus (0.62/0.49), Charolais (0.85/0.53) and Simmentaler (0.73/0.46). It can be concluded that in the case of a weaner calf production system, the Afrikaner (0.60), Bonsmara (0.59), Angus (0.59), Brahman (0.61), and Brangus (0.62) breeds have low methane intensities and can be regarded as environmentally friendly. In the case of an ox production system, the Bonsmara (0.52), Angus (0.50), Hereford (0.52) Brangus (0.49) and Simmentaler have low methane intensities. These values are model predictions and comparing them with measured emissions in South Africa will help determine the accuracy and applicability of these methodologies, ensuring that assessments are tailored to the region's specific environmental and management contexts.

151 Holos – a Canadian whole-farm model to estimate greenhouse gas emissions

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The Holos model is Agriculture and Agri-Food Canada's whole-farm model to calculate greenhouse gas (GHG) emissions including methane (CH₄), nitrous oxide (N₂O) and carbon dioxide (CO₂) from Canadian farms on the basis of National GHG inventory calculations. The model uses Canada-specific Tier 2 emission factors where available (*i.e.*, N₂O, enteric CH₄, soil carbon change) while relying on global Tier 1 emission factors for monogastric animal groups where local emission factors are lacking.

The model supplies default data for all inputs on the basis of national databases. However, the model does not include productivity forecasts (*i.e.*, crop yield and animal weight gain), as it is assumed that farmers have such data readily available, and also because a calibration requirement would prevent model use by most farmers. Although the model is designed for Canada, the model is being adopted in other countries including Norway (HolosNOR) and Ireland (HolosIE).

By applying the model on a representative beef production system (under the lead of Dr. K. Beauchemin) in the Canadian province of Alberta, it was determined that roughly 80% of emissions from the Canadian beef industry stem from the cow-calf production stage, and that enteric CH₄ accounted for 63% of all GHG emissions and N₂O a further 27%. Furthermore, analyzing the Canadian beef industry on a national scale and comparing the status in 1981 vs 2011 (on the basis of Census of Agriculture survey information), we found that the carbon intensity of beef production had decreased by about 14% (with a 14% decline in CH₄, 15% decline in N₂O, and 12% decline in CO₂ for the same amount of slaughter weight), while the contribution of enteric CH₄ remained at 73% of the total GHG budget in both years. The changes occurred due to increased average daily gain and slaughter weight, improved reproduction, reduced time to slaughter, increased crop yields and a shift toward high-grain diets (that improve weight gain, but also lower enteric CH₄ emissions per kg of feed uptake).

Turning our attention from 'What is?' to rather 'What if?', the model played a role to further explore how management practice changes could influence the GHG contributions of the cow-calf production stage, the largest emitter of the beef production cycle. For this purpose, we compared light vs heavy continuous grazing, and with moderate rotational grazing for backgrounding animals as additional factor. Soil carbon change in consequence to the grazing system was modelled using the Introductory Carbon Balance Model (ICBM) within the Holos model. Heavy continuous grazing decreased GHG intensity by 9.2% (due to the higher production efficiency and forage quality), but the use of rotational grazing for backgrounders did not yield any further improvement. However, when accounting for soil carbon change, the GHG intensity estimates decreased by 12-25%, with the lightly grazed systems sequestering roughly double the carbon of the heavily grazed systems.

Upcoming research will further explore the effects of grazing management on soil carbon change and pasture quality, aiming to provide better regionalized estimates to Holos users across Canada and possibly globally.

185 From static to dynamic: Advancing herd modelling in GLEAM-X to support decision making

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The identification of key areas of intervention is crucial to support the transition towards sustainable livestock systems. Effective prioritization of those interventions relies on robust models capable of projecting long-term trends on production, herd size and environmental impacts over time under Business-As-Usual (BAU) and alternative scenarios. The Global Livestock Environmental Assessment Model (GLEAM) supports the sector's stakeholders in identifying hotspots of greenhouse gas (GHG) emissions reduction for livestock agrifood systems. Using a Tier 2 approach, GLEAM performs cohort-level calculations, requiring detailed herd structure data. When primary data is unavailable, GLEAM estimates herd structure through its herd module. Currently, GLEAM's herd module operates as a static model, simulating herd structure over a single year. While effective for annual estimates, it cannot accommodate seasonal variations and long-term projections with changing herd sizes, and changes in the composition resulting from interventions. We present a novel approach to herd modelling that overcomes these limitations and will be part of the advancements in the next generation of GLEAM (GLEAM-X). This dynamic herd module provides greater temporal flexibility, enabling projections of herd size, structure, and production under BAU scenarios, alongside estimates of GHG emissions over time based on evolving herd dynamics. It also allows the evaluation of long-term impacts of herd-level interventions, such as changes in mortality and offtake rates, and the adoption of advanced reproductive technologies like sexed semen and artificial insemination. This capability is essential for identifying interventions that enhance productivity, mitigate emissions, and support climate-resilient livestock systems. The development of the dynamic herd module represents a transformative step in GLEAM's evolution. Incorporating temporal and structural flexibility will provide policymakers and stakeholders with critical insights to prioritize long-term investments, balance productivity with environmental sustainability, and implement evidence-based strategies for a sustainable future in the livestock sector.

187 GLEAM-X: A web application to assess emissions and other environmental impacts for sustainable livestock transformation

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Livestock play a crucial role in providing high-quality nutrition and supporting the livelihoods and resilience of millions of families and communities, particularly in rural areas. In 2015, the global livestock agrifood systems, consisting of farm gate, LUC and supply chain processes annually produced approximately 810 million tonnes (**Mt**) of milk, 78 Mt of eggs, and 330 Mt of meat, providing around 85 Mt of high-quality protein. This global production of animal protein is associated with 6.2 Gt of CO₂ eq emissions, representing about 12 percent of the estimated 50 to 52 Gt CO₂ eq of total anthropogenic emissions in 2015. Achieving sustainable livestock transformation towards lower emissions has become a critical priority, given its roles in mitigating climate change while ensuring food security and advancing global development goals. To assist countries and decision makers in meeting this challenge, the Global Livestock Environmental Assessment Model, developed by the Food and Agriculture organization of the United Nations (**FAO**), has been upgraded to GLEAM-X, a new generation web application offering more advanced functionalities to assess mitigation and other environmental impact indicators. GLEAM-X leverages a scientifically robust and transparent approach, supported by an open data policy. Its highly detailed data enables advanced insights at global, regional, national, and sub-national levels. A dynamic new Herd module has been developed, transitioning from a static model to one that simulates the effects of interventions over time, supporting long-term decision-making. The Feed module has undergone major improvements, with refined greenhouse gas (**GHG**) emission factors for feed production and an expanded list of feed items, reflecting the diversity of countries of origin and supply chains. The model now includes advanced calculations of GHG emissions from manure, incorporating the environmental context of its production, management, and application. A new soil carbon sequestration module has also been integrated, enabling users to assess changes in soil carbon stocks resulting from pasture and rangeland management interventions in grazing systems. Post-farm GHG emissions, including transport, primary processing, and packaging, have been updated through collaborative data collection and energy mix analysis, ensuring more accurate emission factor estimations. To enhance the quality and reliability of results, GLEAM-X now features a sensitivity analysis to identify the most influential parameters and emission factors, automating uncertainty estimation which significantly supports countries in reporting their climate commitments. After a presentation of the key approaches and the concept of the tool, an online demonstration of GLEAM-X will highlight how users can interactively integrate their own data, design mitigation scenarios, and visualize and export the results. By generating UNFCCC-compliant reports and providing more entry points for designing mitigation strategies and simulating impactful scenarios, GLEAM-X strengthens FAO's capacity to support countries in the livestock sector by enabling access to climate finance, improving the reporting of livestock supply chain emissions, and is available for use by diverse stakeholders. We will present the key approaches and concepts of the tool, the architecture of the application and demonstrate how users can interactively design scenarios of mitigation and generate reports for reporting.

198 Opportunities and timeframes to reduce net farm emissions in pasture-based dairy production systems

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Greenhouse gas (**GHG**) emissions from dairying represent a substantial environmental challenge, with mitigation strategies presenting promising solutions at the farm level. This study explored the potential for GHG reductions using an Australian grazing-based dairy farm by modelling the implementation of current and emerging strategies. The study considered the importance of the timing of emissions reduction in the future based on each mitigation strategy's commercial availability and the gradual introduction into farm systems. This study modeled a southeast Australian dairy farm with 720 milking cows, seasonal calving, and a feeding system predominantly based on perennial ryegrass (*Lolium perenne*) grazing. In addition to grazing, each cow was fed 1.8 tonnes of dry matter in grain annually. Manure from the milking parlor was washed and stored in uncovered ponds on the farm. The total nitrogen (**N**) fertilizer application across the farm was 345 kg N/ha/year. Data from 2021 (baseline year), including monthly milk production, feeding practices, herd management, manure handling, and input use, were collected. The GHG emissions profile was estimated using the Dairy Greenhouse Gas Accounting Framework (**D-GAF**) tool (version 14.6). The FullCAM (2020 version) model was used to estimate tree carbon sequestration. Six mitigation strategies were modeled: introducing high condensed-tannin (**HiCT**) white clover (*Trifolium repens*) to existing ryegrass-only pastures, pulse-dosing with 3-nitrooxypropanol (**3-NOP**) at milking, breeding for low-emission cows, planting trees, using enhanced-efficiency fertilizers, and adopting EcoPond technology. Implementation of mitigation strategies was phased in from 2025 onwards based on the projected timeline of commercial availability for each option. Strategies were considered as individual options and as stacks of complementary strategies. The base-year net farm emissions (**NFE**) were 3879 t CO₂e, with enteric methane (**CH₄**) accounting for 58% and CH₄ emissions from manure management contributing 14%. The farm sequestered 163.4 t CO₂e from trees, and the base emission intensity was 14 kg CO₂e/kg Milk Solid (**MS**). Initially, the contributions to emission reductions were minimal as the strategies were gradually introduced, and not all strategies were available for commercial use at the start of the modelling period. Emission reductions begin to accumulate predominantly by 2030 with a 45% reduction in NFE, with all strategies actively contributing by this time. Peak reductions were achieved by 2043, with a total reduction of 3,252 t CO₂e per year, representing an 84% decrease of NFE from the 2021 baseline and an emission intensity of 2.3 kg CO₂e/kg MS. The enteric CH₄ emissions reduction that occurred at the peak year was 27%. HiCT white clover, followed by breeding, and 3-NOP contributed to this by reducing 357, 228, and 155 t CO₂e per year, respectively. EcoPond reduced emissions by 415 t CO₂e/year, while tree planting reached the peak sequestration of 2,076 t CO₂e/year in 2043. Breeding for low-emission cows achieved its maximum impact of 355 t CO₂e/year by 2058, becoming a permanent trait in the herd. Overall, this study highlights the importance of adapting the mitigation measures without delay in reaching targets.

205 Leveraging breed-specific data to guide targeted greenhouse gas mitigation strategies in Uganda's dairy sector

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Dairy production plays a vital role in Uganda, supporting 6.8 million households and contributing 17% to the country's total agricultural gross domestic product. This subsector is crucial in the central region of Uganda, where over 59.8% of the population is engaged in livestock production. Despite its potential, dairy production in the central region faces many challenges, including low productivity, soil infertility, and limited resources, particularly land, in the smallholder farming systems. Furthermore, dairy production practices are under scrutiny due to their increasing carbon footprint, which has directly impacted the sector, leading to more frequent instances of climate variability. To evaluate the extent of these issues and propose priority dairy interventions, we conducted a case study in the Mpigi and Nakasongola districts, two key cattle corridors in the central region. This study aimed at evaluating the impact of the current dairy production practices on breed-specific greenhouse gas emissions (GHGe) and the likely changes due to the adoption of the bundled dairy husbandry interventions (animal health, genetics, feeds and forages) proposed by the research partners in the study sites. Using I-CLEANED an ex-ante environmental footprint calculator for livestock systems, we evaluated the traditional (Ankole and Nganda) and exotic breeds (Friesian, Ayrshire, Guernsey, Jersey) in seven villages across the study sites. Data for parameterization was obtained from the AADGG genetic gains platform, including information on breeds, milk production, weight, lactation periods, and feed baskets from 1,500 dairy farmers across two districts. This was further complemented by data from the Gendered Feed Assessment Survey of 2023, which focused on crop production, livestock, and feed resource availability. Additionally, information on feed nutritive values, agroclimatic conditions, and weight gains was gathered from secondary sources. To validate the data on feed intake, milk production, and crop inputs, follow-up interviews were conducted with farmers. The selection of breeds and villages in this study was based on the prevalence of breeds across multiple villages and the completeness of data obtained after a rigorous validation process. Baseline results indicated that Guernsey had the highest GHGe intensity with an average of 3.198 kg CO₂ eq. kg⁻¹ FPCM among the villages. In Nakasongola, Friesians and Guernsey emitted 2.07 and 7.77 kg CO₂ eq. kg⁻¹ FPCM respectively which was higher compared to Mpigi district (1.68 and 2.055 kg CO₂ eq. kg⁻¹ FPCM, respectively). However, with the simulation of bundled interventions, variations in GHGe intensity levels were noticeable with greater improvements observed in Mpigi district compared to Nakasongola district. GHGe emission intensity gains varied between 1 to 1.87 kg CO₂ eq. kg⁻¹ FPCM for all breeds. Differences in feed quality and availability among villages and districts affect the emission levels of dairy production systems. A reduction in natural pasture intake, enhanced animal health and welfare, and a gradual integration of improved breeds in Nakasongola could help transition the district into a low emitter and a net supplier of milk and related products in the region. Improving feed conversion efficiency and reproductive performance among the predominantly exotic breeds in Mpigi have immediate mitigation gains.

240 Methane emissions from Bonsmara cattle in feedlot and grass-fed production systems

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Methane emissions from cattle are generated through enteric fermentation, a natural digestive process in ruminants, as well as through manure, depending on the manure management practices. In South Africa, most cows spend their lives on natural rangelands. Calves are typically weaned at 6-8 months when they are sold (except for the replacement heifers). Most calves go to feedlots, while a few will remain on natural rangelands. Feedlots dominate the industry, contributing to approximately 75% of the country's beef production. Feedlots are characterized by high-energy grain diets, whereas grass-finished cattle remain on forage-based diets for an additional period beyond the original weaning period. Bonsmara cattle, renowned for their adaptability to both finishing systems, offer an ideal model for evaluating these differences. This breed is an efficient producer and known for its superior meat quality. This study employed the Intergovernmental Panel on Climate Change (IPCC) Tier 2 methodology to estimate and compare methane emission factors (MEFs) from enteric fermentation and manure management in Bonsmara cattle finished in feedlot (n = 19) and grass-fed (n = 212) production systems. Lower enteric MEFs were estimated for feedlot cattle compared to their grass-fed counterparts. When expressed per unit of weight gain, enteric MEFs were nearly four times lower for feedlot cattle (0.046 versus 0.171 kg methane/kg gain). These results corroborate existing literature, which suggests that feedlot systems have lower enteric methane emissions than grass-fed systems, primarily due to differences in feed quality and growth rate. It should be noted that possible carbon sequestration in rangelands and the carbon footprint of feed production for feedlots were not included in this comparison. In terms of the manure MEFs, feedlot cattle had higher manure MEFs than grass-fed animals. The kg methane emissions per Large Stock Unit (LSU) during the growing period was 0.050 kg (feedlot) versus 0.0043 kg (grass-fed), an almost 12 times difference. (LSU is the equivalent of an ox of 450kg and similar to an Animal Unit in the USA). This difference is attributed to differences in manure management, since feedlot operations facilitate anaerobic digestion, which results in increased methane production. Literature supports the observation of higher manure MEFs in feedlot compared to grass-fed systems. The refinement of methodologies to estimate methane emissions is essential to better account for local factors influencing methane emissions across beef production systems. While frameworks like the IPCC guidelines provide valuable guidance, there is a need for South African studies to evaluate how accurately these equations model actual methane emissions under local conditions. Comparing model predictions with measured emissions in South Africa will help determine the accuracy and applicability of these methodologies, ensuring that assessments are tailored to the region's specific environmental and management contexts. Future research could be expanded to include diverse geographical areas, a range of cattle breeds, and various production systems across South Africa. Moving beyond reliance on default IPCC values and incorporating country-specific data and methods would likely enhance the accuracy of emission estimates.

247 The GRA Manure Management Network: presenting research priorities for manure management in a changing world

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The Manure Management Network (**MMN**) is a forum under the Global Research Alliance on Agricultural Greenhouse Gases (**GHG**), set to support worldwide collaborative research for reducing GHG emissions from livestock production, increasing manure nutrient use efficiency and manure contribution to soil health, within a circular economy. Current priorities in the search for environmentally friendly and efficient manure recycling, and MMN responses to these challenges are presented below.

One priority is linked to the growing number of livestock in the Global South. For example, increased incomes and population growth across Africa have led to higher consumption of livestock products. This is changing livestock production systems from smallholder and pastoral systems where animals are often free to graze to confined operations with more livestock inside housing or open confinements (e.g. feedlots) most of the time. This is increasing the amounts of manure in regions where there is not a strong tradition of intensively managing manure and that often lacks efficient management systems. This can increase the risk of greater GHG and ammonia emissions as well as leaching losses. Another priority should be how best to quantify emissions. Worldwide, scientists measure gaseous emissions from manure using different measuring techniques that are not always directly comparable. Because of this disparity, results cannot be easily combined to parameterize emission models or calculate emission factors (**EFs**). This calls for an “intercalibration study” comparing and harmonizing different measurement technologies. This should also include information on supporting measurements (e.g., soil, weather, manure characteristics) required for developing and running predictive models. The third priority should be solid manure management. This practice is commonly used globally and is becoming more important in the Global South. However, there are few studies of GHG emissions from this management chain, and EFs of GHG, NO_x and NH₃ are highly variable reflecting the multitude of factors that can affect decomposition and loss pathways during solid manure storage. More measurements are needed to accurately represent this category in emissions inventories. Finally, we need improved predictive models describing CH₄ and N₂O emissions from both in-house and outside stores. Specifically, our aim is to calibrate and validate process-based models. Regarding CH₄ emission from stored liquid manure there is a need for improved understanding of dietary and livestock effects on B₀ (i.e. the maximum amount of CH₄ that can be produced from manure), as well as an improved understanding of CH₄ production kinetics from stored liquid manure to help in the development of predictive models of CH₄ emissions.

To address these priorities, the MMN intends to call for collaboration through meetings, webinars, and a joint effort to address the following: 1) provide information about livestock farming in Africa using information from reports, peer-reviewed articles and grey literature; 2) standardize methods to measure gas emissions from livestock barns, manure stores, composting, and applied manure; 3) determine gaseous emissions from the solid manure management chain; 4) improve our understanding of B₀ and the kinetics of CH₄ production in biogas production and from stored liquid manure.

263 How to better improve tier methodologie's accuracy for methane emissions factor in Sub Saharan Africa?

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Enteric methane emissions (**eCH₄**) from ruminants are the main source of greenhouse gas (**GHG**) from the livestock sector in sub-Saharan Africa (**SSA**). The SSA countries face significant challenges in assessing their climate commitments in the livestock sector, particularly in terms of mitigation options. This is due to the lack of GHG accurate data obtained through *in vivo* measurements. To do this gas inventory, African countries use moreover IPCC Tier 2 methodology. The objective of this study was to evaluate the performance of this methodology in SSA by comparing gross energy intake (**GEI**), dry matter intake (**DMI**), and emissions factors (IPCC Tier 2 estimation vs direct measurement) using actual data collected in within this region. The experiments took into account the diversity in SSA of local both resources (natural rangeland fodder - green and hay, cultivated forages - green and hay, cereal and legume crop coproducts – hay, legume tree forage – hay), feeding practices (exclusive natural rangeland fodder from each season or cultivated forage, supplementation with crop coproducts and legume tree forages), cattle breed and category (Fulani zebu steers, bulls and suckler cow), and seasonal variation of feed accessibility (varying the quantity of the feed offered in the wet season, cold dry season, hot dry season). The collected data grouped body weight (weekly), intake (daily refusal collection), digestibility (daily total faeces collection) and eCH₄ emissions (GreenFeed® system). Data collection lasted 2.5 years and included 35 trials (feeds). Each trial lasted 3 weeks split into 2 weeks of diet adaptation and 1 week of data collection. The data were used to calculate the GEI, DMI according to IPCC (2019) Tier 2 procedure. Those GEI and DMI obtained were integrated in Tier 2 emissions factors (**EF**) equations (based on GEI and DMI, respectively). The eCH₄ yield (**MY**) were deducted from EF. The comparison of means (Tier 2 vs actuals data) shows significant difference per Tropical livestock Unit (**TLU**) ($P < 0.001$) for GEI (139.49 vs 89.23 MJ/day), DMI (7.90 vs 4.93 kg/day), emissions factors (64.04, 67.16, and 46.44 kg/year for equation 1 based on GEI, equation 2 based on DMI, and actual data, respectively). Consequently, YM (22.23, 23.30, and 26.68 g/kg DMI per day were obtained for equation 1, equation 2, and actual data, respectively). These direct eCH₄ measurements, coupled with dietary data collection considering local resources, feeding practices, cattle breed and category, availability of resources and seasonal variation in SSA, will help implement the IPCC Tier 2 methodology to accurately estimate livestock emission factors in this region.

376 A GIS-based LCA approach for quantifying greenhouse gas emissions in Malaysian commercial dairy farms

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The livestock sector is responsible for approximately 14.5% of global greenhouse gas (**GHG**) emissions. Within this sector, dairy and beef cattle represent the largest share, accounting for roughly 65% of emissions, followed by small ruminants and non-ruminants. Dairy farming contributes significantly to global climate change through GHG emissions, primarily methane (**CH₄**) and nitrous oxide (**N₂O**) from animal husbandry and manure management. In Malaysia, the dairy sector faces critical gaps in awareness, region-specific data, and mitigation strategies to address the impact of GHG emissions, hindering progress toward sustainability and **ESG** (Environmental, Social, and Governance) goals. To bridge this gap, this study employs a GIS-integrated Life Cycle Assessment (**LCA**) approach to quantify GHG emissions from a commercial dairy farm, adopting a cradle-to-farm-gate system boundary and a functional unit of 1 kg CO₂-eq per kg live weight (kg CO₂-eq/kg LW). The research focused on an 838-acre farm with 4,350 cattle, comprising 1,488 lactating cows, 1,557 gestating cows, and 574 dry cows, averaging 20 liters of daily milk production per cow. The LCA model estimates emissions across three scopes: *Scope 1* (direct on-farm emissions: manure management, farm operations; indirect on-farm emissions: enteric fermentation based on feed diet inventory estimation), *Scope 2* (indirect emissions: electricity, fuel, and water consumption), and *Scope 3* (upstream/downstream emissions: feed production, transportation, and pharmaceuticals). Field data collection for *Scope 1* included direct gas and manure sampling at animal pens and waste treatment areas, with 64 gas samples (analyzed via Gas Chromatography Thermal Conductivity Detector (**GC-TCD**) for CO₂ and CH₄) and 90 manure samples (for nitrogen and volatile solids analysis) to be analyzed. Farm inventory data for *Scopes 2* and *3* were also acquired, with GHG emission calculations performed using Intergovernmental Panel on Climate Change (**IPCC**) guidelines. All data and results will be used to estimate total GHG emissions and will be integrated into a Geographic Information System (**GIS**) database for LCA modelling, enabling spatial visualization and mapping of emission hotspots. This GIS-based LCA model will provide detailed visualizations of emission sources, facilitating efficient tracking and mitigation strategies. Consequently, this approach identifies key contributors to the farm's carbon intensity and supports Malaysia's commitment to achieving net-zero emissions under global climate agreements. By addressing data gaps and offering actionable insights, this study aims to advance sustainable practices in Malaysia's dairy sector while providing a replicable framework for similar regions.

*This research is ongoing; final findings (results and discussion) will be completed and shared at the conference.

444 Advancing institutional engagement for sustainable livestock production in Pampas region of Argentina: challenges and opportunities

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This study evaluates an INTA-led multi-stakeholder platform engaging producers, extension agents, researchers, and institutions to enhance the sustainability of pasture-based beef production in Argentina's Pampas. The initiative fosters collaboration to identify limiting factors and co-develop tailored strategies with farmers.

Data were collected from 36 livestock farms – 22 cow-calf, 4 cow-calf–stocker, and 11 full-cycle – averaging 763 ha (range: 110–1700) and a stocking rate of 0.74 LU/ha. Detailed surveys on herd structure, feeding, grazing, land use, and manure management informed a locally adapted carbon footprint (**CF**) calculator aligned with IPCC 2019 guidelines. Emissions included enteric methane, manure, soil N₂O, and energy-related CO₂.

Cow-calf farms (738 ha avg., 74% native grassland) produced 99.5 kg liveweight (**LW**)/ha/year (range: 62–150), with a mean CF of 20.9 kg CO₂e/kg LW (range: 16.9–25.9). Despite similar weaning rates (**WR**, ~80%, range: 65–91.2%), CF varied by up to 7 kg CO₂e/kg LW, indicating that reproductive performance alone does not explain efficiency gaps. Key drivers included grazing intensity, diet, replacement strategies, and cycle length. Stock efficiency (**SE**, weaned calf at 6 months/cow weight) was critical: increasing SE from ~0.2 to >0.3 reduced CF by ~40% (from 28 to 16 kg CO₂e/kg LW), underscoring the role of consistent management.

Cow-calf–stocker farms (653 ha avg., 64% native grassland) achieved 147 kg LW/ha/year (range: 107–192), with 83.9% WR and stable gains during rearing. Average daily gain (**ADG**) was 0.48 kg/head/day (0.55 for males, 0.42 for females). Mean CF was 16.1 kg CO₂e/kg LW, with a 30% range across farms, driven by forage quality, management, and rearing efficiency. As in cow-calf farms, higher SE was linked to lower CF.

Full-cycle farms (1456 ha avg.) yielded 152 kg LW/ha/year (range: 102–231) with an average CF of 17.6 kg CO₂e/kg LW (range: 15.1–23.2), largely influenced by rearing-phase performance. Farms with ADG >600 g/day showed CFs of 15–16 kg CO₂e/kg LW, while those <450 g/day exceeded 20 kg CO₂e/kg LW. Male ADG was 0.55 kg/day (rearing) and 1.19 (finishing); females reached 0.46 and 1.12, respectively. Final LWs averaged 424 kg (steers) and 362 kg (heifers). Unlike rearing, finishing phase factors (e.g., final weight, duration) had limited CF impact.

The platform's participatory approach promoted trust, knowledge exchange, and practical improvements through personalized feedback. It enabled data-driven decisions, identified emission hotspots, and co-designed strategies using local metrics. Replicating this approach in other regions may scale its impact, contributing to a more resilient and low-emission livestock sector in Argentina through ongoing collaboration and innovation.

448 Challenges in assessing the climate impact from extensive ruminant livestock systems

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Grassland-based livestock systems contribute significantly to global greenhouse gas (GHG) emissions, accounting for approximately 26% to 34% of total GHG emissions from livestock, according to FAO reports from 2022 and 2013, respectively. Furthermore, the GHG intensity and carbon footprint of extensive systems are generally estimated to be higher than those of more intensive systems. Some research has indicated that livestock intensification can reduce GHG intensity and carbon footprint, primarily due to improvements in reproductive management, increased feed quality, and the use of more productive breeds. However, this intensification can also lead to a shift in both the types of gases emitted and the sources of those emissions. For example, when measured per kilogram of meat or milk, intensification has been shown to result in lower enteric methane (CH₄) emissions and reduced nitrous oxide (N₂O) emissions from soil at the farm level. However, it increases emissions from manure, particularly CH₄, and pre-farm gate carbon dioxide (CO₂) emissions due to the indirect use of fossil fuels in feed production. Carbon dioxide emissions from land use change are associated with both intensive (indirectly through purchased feed) and extensive systems (such as deforested areas converted into pastures for livestock).

Extensive livestock systems are characterized by several features that differ significantly from more intensive systems. These systems are generally diverse, encompassing a variety of plant species and animal breeds, and are interconnected with biologically diverse environments. Consequently, the interactions between soil, plant, and animal components are complex and influenced by numerous ecological processes, often resembling natural or semi-natural ecological dynamics. This complexity poses significant challenges when attempting to use generic approaches to accurately quantify their true climate impact, as it involves processes other than those connected merely with GHG emissions (e.g. changes in albedo, fire risk, and carbon change dynamics). Furthermore, extensive livestock systems occupy and utilize the same areas – such as marginal land and semi-natural grasslands – previously inhabited by wild herbivores. As a result, the GHG and nitrogen (N) emissions from current extensive livestock practices, driven by human activity, partially replace the emissions that wild animals naturally produced.

Here, we provide evidence and discuss the challenges of quantifying climate impacts in extensive ruminant livestock systems. Our focus is on the uncertainties associated with: (i) the use of generic and linear emission factor (EF)-based approaches (e.g. IPCC-based) for quantifying GHG and N emissions – such as enteric methane CH₄, manure nitrous oxide N₂O and ammonia (NH₃), and soil N₂O – and soil organic carbon (SOC) changes; and (ii) the assumption that 100% of current GHG emissions from extensive systems are solely the result of human actions, rather than part of the natural baseline conditions of reference ecosystems. We compared the climate impact results from a selection of case studies using IPCC-based approaches – assuming that 100% of emissions drive climate change (i.e., they do not replace any previous emissions) – with those obtained from non-EF-based simulation models that estimate GHG and N emissions and considering the GHG emissions under on-site natural conditions.

460 The Ruminant Farm Systems (RuFaS) model allows evaluation of whole-farm greenhouse gas emissions

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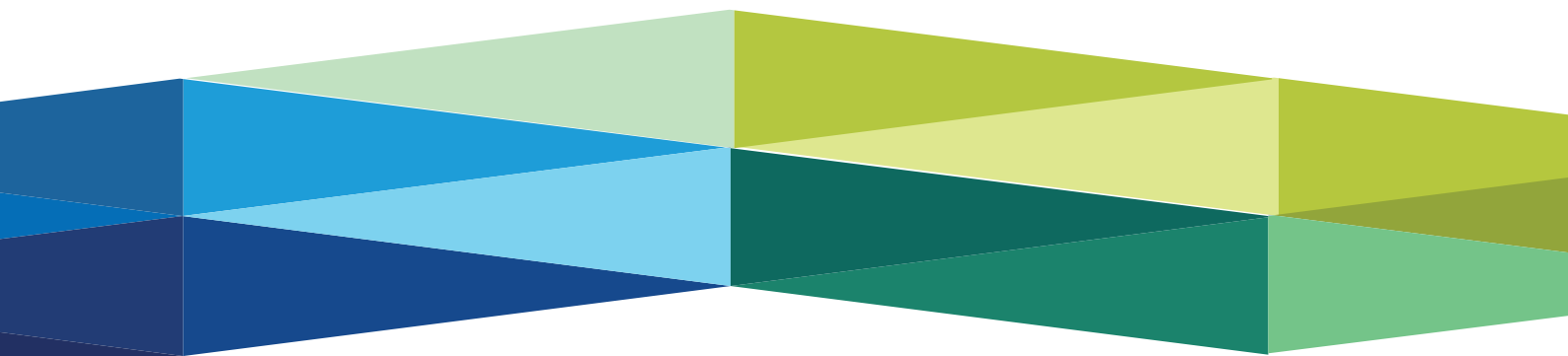
Modern dairy farms are complex systems that require sophisticated analysis and management to meet production, profitability, and environmental stewardship goals. Dairy farmers, their advisors, and other dairy value chain stakeholders need systems-level information and insights to balance potentially conflicting objectives that are frequently difficult to understand when analyzed in isolation. The Ruminant Farm Systems (**RuFaS**) model is a modular, process-based, whole farm simulation platform designed to optimize dairy farm production and quantify economic and environmental outcomes, including greenhouse gas emissions. Whole-farm systems models can be particularly useful for analyzing interactions between different farm components and providing system-level insights, since they can quickly and cost-effectively evaluate various farm types and management practices that are either impossible or impractical to study through experimentation. RuFaS integrates four primary biological and physical modules to simulate dairy farm management of soils and crops, feeds, animals, and their manure. Each module simulates a farm system component by synthesizing knowledge from the scientific literature into mechanistic models when available or by relying on empirical models when needed. By simulating the connections between the biological and physical processes in the farm and incorporating stochasticity, RuFaS generates unique results based on specific management decisions, thus offering insights that simpler models cannot provide. A key feature of RuFaS is its ability to simulate the interaction of processes across the entire farm and how management decisions affect whole-farm production, economic, and environmental outcomes. For example, RuFaS simulates manure production by animals, its collection, treatment, storage, and soil application for its subsequent use in growing crops that are then harvested and fed to the animals. The results are detailed through every step, to separately quantify variables of interest, such as carbon dioxide, methane and nitrous oxide emissions at each farm source. This comprehensive approach ensures that all outcomes are fully documented and accounted for in the simulation, including those influenced by stochastic elements. Post-simulation, RuFaS employs additional processes to evaluate energy use and profitability, and aggregate emissions to generate user-customizable reports, enabling detailed scenario analyses. This allows users to assess the current state of a farm and explore various management options and their potential impacts. Built-in sensitivity analysis capabilities further enhance the platform's utility by identifying key drivers for each scenario. RuFaS is open-source, transparent, and follows well-defined documentation standards, encouraging contributions from the broader scientific community. Its architecture, developed using modern software engineering principles, supports regular evaluation, improvement, and expansion to meet the needs of researchers, farmers, and dairy supply stakeholders. Overall, RuFaS provides an integrative and accessible platform for exploring on-farm decision-making and evaluating critical outcomes.



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Theme 8: National greenhouse gas inventory and policy frameworks with carbon finance mechanisms



35 Allocating enteric methane (CH₄) emissions from poultry supply chains using the FAO guide

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The guide *Greenhouse Gas Emissions and Fossil Energy Use from Poultry Supply Chains* (LEAP, 2016) is a critical reference for modelling poultry emissions, particularly enteric methane (CH₄). While the guide provides emission factors, it lacks detailed instructions for their application, which can lead to inaccurate estimates when modelling inventories that include different phases of animal development. Therefore, as enteric CH₄ emissions are related to the quantity and quality of animal feed, we propose two equations to allocate emissions per phase, considering both nutrition and feed conversion. We adopted weight gain as the allocation parameter, given its strong correlation with quality and quantity of feed. We developed and applied the following equations: i) $Pwg = ((kg_{ph} * N_{an}) * 100) / RV$ – where: Pwg = portion of weight gain in the phase (%); kg_{ph} = kg of live weight/animal/phase (kg); N_{an} = number of animals; RV = reference value (kg); ii) $EM_{Ph} = EM_{sys} * (Pwg / 100)$ – where: EM_{Ph} = enteric CH₄ emitted by phase; EM_{sys} = enteric CH₄ emitted by the production system (all phases), in kg; Pwg = portion of weight gain in the phase (%). The results indicate that allocating enteric CH₄ emissions based on feed conversion ratio (FCR) - which encompasses two fundamental parameters of animal nutrition; weight gain efficiency and emission mitigation efficiency - are reflected in the allocated values. The literature emphasizes the importance of nutrition models that incorporate complementary methods to improve the estimation of enteric CH₄ emissions. Some models of nutrition can incorporate different and complementary methods for predictability purposes, those based on food intake, proportions and compositions and characteristics of animals, are seen by the scientific community as crucial in predicting the production and emission of enteric methane. Therefore, adopting complementary allocation models that consider the feed conversion ratio (and its parameters), for models in already established guides, such as those from the IPCC (2006) present in the LEAP guide (2016) seems to be a prudent path to follow.

84 An estimation of greenhouse gas emissions from livestock in Nepal

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This study estimates and projects livestock-related greenhouse gas (**GHG**) emissions in Nepal between 2002 and 2022, providing crucial insights for climate change mitigation strategies. The latest secondary data on livestock population was collected from the Ministry of Agriculture and Livestock Development (**MoALD**) Nepal. The IPCC tier 1 methods were used to estimate emissions and forecast future trends. Studies reveal that Nepal's livestock-related GHG emissions reached 31,918 Gg CO₂e /year in 2022, with buffalo accounting for 39 % of the total emissions followed by cattle and goats. In 2022, the primary sources of emissions were direct nitrous oxide (48.2%), enteric methane (44.7%), manure methane (4.5%), and indirect nitrous oxide (0.6%). Future projections indicate a potential increase in total GHG emissions by 3.06 % and 3.56% up to 2050, suggesting a growing environmental impact if current practices continue. The provincial (regional) analysis identified Koshi province as the highest emitter in 2022. This research underscores the need for effective management strategies to mitigate emissions from the livestock sector in Nepal. Further, it also recommends transitioning to the IPCC Tier 2 approach when sufficient national-level data becomes available to enhance the accuracy of future inventories.

92 Distribution of greenhouse gas (GHG) emissions from cattle in top ruminant-producing African countries

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The livestock sector is a significant contributor to greenhouse gas (**GHG**) emissions in Africa accounting for about 30% of the continent's total emissions. Cattle production is a major source of these emissions due to enteric fermentation and manure management. This study examines the distribution of GHG emissions from cattle in the top 10 cattle ruminant-producing African countries, which account for over 70% of the continents' ruminant population (approximately 280 million head). The study utilized data from the Food and Agriculture Organization (**FAO**) of the United Nations, the Intergovernmental Panel on Climate Change (**IPCC**), and national reports from the studied countries. The GHG emissions from cattle were estimated using the IPCC Tier 1 methodology, which considers emissions from enteric fermentation, manure management, and other sources. The results show that the top three emitting countries are Nigeria (19.55 Mt CO₂e), Ethiopia (16.22 Mt CO₂e), and South Africa (12.09 Mt CO₂e). These countries account for over 50% of the total GHG emissions from cattle in the studied countries. Methane emissions from enteric fermentation and manure management are the dominant sources of GHG emissions, accounting for 60-80% of total emissions. Carbon dioxide emissions from feed production and land use change are also significant contributors. The study highlights the need for targeted mitigation strategies to reduce GHG emissions from cattle production in these countries. Improving feed quality and grazing practices, enhancing manure management, and promoting more efficient breeding practices can help reduce emissions. Additionally, the study emphasizes the importance of considering the regional context and specific circumstances of each country when developing mitigation strategies. This includes taking into account factors such as livestock population, feed availability, and cultural practices.

115 Inventory of greenhouse gas emissions for cattle in Zambia using the Tier 2 approach

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The present study estimated annual greenhouse gas emissions from cattle in Zambia from 1994-2022 using the IPCC Tier 2 approach which enhances accuracy through reflecting country specific circumstances and monitoring of the effect of policies and interventions which is lacking in Tier 1. Comprehensive literature review was undertaken to obtain country-specific activity data including feed basket composition, nutritional quality of feeds, and manure management practices. The FAOSTATS database was the main source for the cattle population in Zambia with dairy cattle population representing 9.1%. Due to data gaps identified, an expert judgement workshop with 14 livestock production experts was convened. Cattle production systems identified were for dairy (commercial and emergent) and for other cattle (commercial, emergent and extensive/traditional). Five sub-categories were selected for each cattle production system namely: adult bulls, adult cows, young bulls/steers, heifers, and calves. However, feedlot and oxen subcategories were added for the beef commercial and extensive/traditional production systems respectively. Six manure management systems (i.e., liquid/slurry, dry lot, solid storage, burned for fuel, biogas digester and pasture/range/paddock) were the practices in Zambia. Secondary data collected were summarized to obtain means and variances. Missing emission factors (EF) in intervening years were calculated using interpolation and extrapolation accordingly. Appropriate equations according to IPCC guidelines were employed in determining the EF and emission estimates for methane and nitrous oxide. The enteric CH₄ emission increased from 165Gg CH₄ in 1994 to 300.2 Gg CH₄ in 2022 with the extensive/traditional and commercial beef production systems contributed the most (i.e., 72.5% and 14.3%, respectively) of the total yearly enteric CH₄ emissions. Dairy cattle contributed an estimated annual 16.75 Gg CH₄ or 8.41% of the total enteric CH₄ emissions with the commercial and emergent dairy production systems accounting for 2.7% and 5.9 % respectively of the total emissions. The implied CH₄ emission factors for dairy cattle in the commercial and emergent increased from 68.2% to 86.7% and from 55.2% to 55.9% respectively over the years. However, for the other cattle, the differences over the years were marginal for both emergent and extensive/traditional beef production systems. The mean CH₄ EF for commercial dairy ranged from 17.8 kg CH₄ head/year for calves to 99.2 kg CH₄/head/year for adult cows and an average of 18.7 kg CH₄ /head/year for calves in the emergent dairy to 70.5 kg CH₄ /head/year. The same scenario was observed for the other cattle except that the magnitude was lower. Generally, adult females had higher enteric methane EF than the adult males while the enteric methane EF increased from 1994 to 2022 mainly driven by population growth. Manure management methane EF were generally higher for dairy cattle than for other cattle. However, total emissions from the manure management system, were higher for other cattle for the period considered due to the higher population size. This study has helped to refine emission estimates earlier obtained using Tier 1 approach, strengthen the national GHG inventories and contribute to the tracking of the livestock sector performance.

165 The Flemish Centre of Expertise for Agriculture and Climate: A multidisciplinary knowledge hub

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Collaboration and discussions between researchers and policy makers is a crucial step in creating impact from climate research. However, creating and maintaining these networks is time consuming. Therefore, having a central coordinating body that supports researchers and acts as a key liaison between the research community and society can be highly beneficial. The ILVO Centre of Expertise for Agriculture & Climate (**ELK**) takes up this role. ELK supports and advises agricultural and environmental policy makers and the broader agricultural sector on climate-related topics. ELK consolidates the multidisciplinary climate knowledge within ILVO, identifies knowledge gaps, and induces new research. It monitors international developments, placing the Flemish context in Belgian, European and global perspectives, with the aim of accelerating the application of research in policy. Today, the ELK encompasses a group of nearly 100 passionate scientists working on a variety of potential climate solutions, ranging from alternatives for soy in feedstuffs, to the implementation of carbon farming. As an example, recently, the Flemish government has agreed on a budget of 25 million euros to support farmers in accelerating carbon farming implementation. The ELK has provided valuable insights towards fine-tuning this policy measure. The ELK's research themes are focussed on two main pillars: reducing the impact of Flemish agriculture on climate change (mitigation) and enabling agriculture to adapt to climate change (adaptation). Adopting a systemic approach, ELK considers broader sustainability aspects of the agri-food chain, encompassing societal, economic, and environmental factors.

As an expression of ELK's multidisciplinary approach, a 'systems thinking' exercise has been performed. This exercise involved experts from diverse research areas collaborating to understand the complex interplay between climate mitigation, climate adaptation, and carbon storage, in an agricultural context. This framework is essential for assessing whether climate measures are genuinely climate-smart, avoiding solutions that achieve one goal but impede others. The ELK systems thinking exercise delves into various key subsystems essential for understanding agricultural and climate issues. These subsystems include social factors (i.e. policy, education, consumer preferences), farmer's choices (i.e. land use, livestock management, crop selection), economic factors (pricing, available technologies, production quantity) environmental factors (soil health, water availability, biodiversity) and climate change (emissions and effects). By elucidating this intricate web of connections, we gain insight into climate mitigation and adaptation factors and how they are intertwined, transcending individual research themes.

The results of this systems thinking exercise and all other ELK research is communicated to a broad audience via a website, a quarterly newsletter and an annual event. The system figures are used today to communicate the complexity of climate research to policy makers and citizens, and are used as a guidance for our researchers when writing new project proposals. This way, projects and policy measures can be worked out that are based on solid research, but are also implementable in practice. In conclusion, the ELK aims to be the central actor for all climate-related research in agriculture in Flanders, providing knowledge and insights to farmers, researchers and civilians alike, and to improve the integration of climate science in policy.

211 The importance of strengthening national capacities for greenhouse gas emissions assessment in the livestock sector

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Greenhouse gas (**GHG**) emissions assessment and Nationally Determined Contributions (**NDCs**) are critical components of global efforts to address climate change under the Paris Agreement. Together, they enable countries to measure, report, and implement climate actions aimed at reducing emissions and limiting global temperature rise to 1.5°C or 2°C above pre-industrial levels. The agriculture and livestock sectors often feature prominently in NDCs due to their significant contribution to GHG emissions, especially in Low- and middle-income countries (**LMIC**). The livestock sector is a significant contributor to global methane and nitrous oxide emissions, making it critical for detailed inventory improvements. Accurate GHG assessments from the livestock sector are essential for reporting. However, developing countries lack accurate, comprehensive data on livestock populations, feed types, and management practices, which are crucial for GHG assessments. Instead, they use the Intergovernmental Panel on Climate Change default emission factors (**EFs**) that might not accurately reflect local livestock production systems, hence the need for country-specific EFs. There are LMIC countries that undertake agricultural or livestock development projects in collaboration with international organizations and may collect livestock data. However, many of these LMIC countries lack technical expertise and institutional capacity for comprehensive GHG assessments and implementation. There is the need for capacity building to these countries to equip them with understanding, skills and access to knowledge that enables them to develop context-specific EFs, allow to explore strategies such as improving feed quality, optimizing herd management and adopting manure management practices to reduce emissions and enhances the ability to engage in global partnerships. With improved GHG assessments, countries may access international funding mechanisms like the Green Climate Fund (**GCF**) for implementing mitigation projects. Reliable GHG assessments enable policymakers to prioritize interventions, set realistic mitigation targets and monitor progress over time. Training and tools can encourage sustainable livestock practices that balance productivity with environmental conservation. The Livestock, Innovation, Climate Change, and Post-harvesting team in the Animal Production and Health Division (**NSA**) of the Food and Agriculture Organization of the United Nations has been conducting training on the use of Global Livestock Environmental Assessment Model (**GLEAM**). The curriculum includes a module for basic knowledge of GHG emissions in the livestock sector and use of the GLEAM Tool and the second module aims at transferring advanced skills that would be used in identifying and testing mitigation packages that can be implemented under the NDC, in livestock project designs and climate finance access. The FAO GLEAM team has conducted in-person training workshops in East African and central Asia among which two of the countries in East Africa have received advanced skills module and also include virtual training session. We will present the curriculum, the lessons learnt, and challenges faced during the training workshops. Countries need the capacity especially with the commitment to the United Nations Framework Convention on Climate Change on reporting the emissions in their NDCs.

301 Estimating the carbon footprint of milk production on Polish farms

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The carbon footprint of milk produced on domestic dairy farms was analyzed as part of a collaborative research network among Agricultural Universities in Poland (SUP-RIM). The study aims to support the environmentally sustainable development of the Polish dairy sector. The Annual Nutrient Cycling Assessment (**ANCA**, Wageningen University & Research Centre) tool was employed to calculate CO₂-equivalents (**CO₂-eq**) per kilogram of fat-and-protein-corrected milk (**FPCM**). The entry data from farms requested detailed information, such as crop and dairy production characteristics, housing system, manure management, energy resources, etc. The study encompassed 46 dairy farms across Poland, ranging from large-scale farms with 1,300 cows to farms with smaller operations (6-100 cows). Average milk production per cow was 9.241 kg, from 3.520 kg in a small extensive farm to 15.000 kilograms in intensive production systems. The carbon footprint of milk produced on the audited farms was characterized by an average of 1.64 kg CO₂-eq/kg FPCM, a median of 1.50, a standard deviation of 0.51 and a variance of 0.27, with values ranging from 1.03 to 4.26. The first quartile (Q1) was 1.43 and the third quartile (Q3) was 1.74, resulting in an interquartile range of 0.31. The most important contributor of emission was ruminal fermentation (39% on average), followed by emissions from imported farm inputs (29% on average). Future efforts will include continued monitoring and the development of a new emission calculator, adjusted to Polish conditions and able to be easily used by the farmers. This tool aims to provide dairy producers with practical solutions for tracking and mitigating the carbon footprint of milk production in Poland.

381 International Methane Emissions Observatory's global engagement in reducing agriculture methane emissions

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The Global Methane Assessment estimates that agriculture accounts for about 40% of anthropogenic methane emissions, the majority of which are from livestock. To achieve reduction in agriculture methane emission, open, measurement-based and actionable data is essential to track changes in emissions over time. As a core implementing partner of the Global Methane Pledge, the UN Environment Programme's International Methane Emissions Observatory (IMEO) exists to provide reliable, public, policy-relevant data to facilitate actions to reduce methane emissions.

The objective of this abstract is to give an overview of the ongoing IMEO's methane baseline studies in Colombia and Nigeria where agricultural methane emissions are a focus area.

We make an initial assessment per country through literature and reports, feed the existing prior to satellite inversion model and apportion the emission to the agriculture sector. We focus on livestock and rice paddy methane emission and design small studies prioritizing the integration of multi-scale measurement methods such as respirometric chambers, polytunnel, eddy-covariance method and drone-based measurements, also accounting for seasonal variability. We incorporate these new measurement data to improve satellite inversion analysis. Further, by combining activity data through agricultural censuses and statistical agencies, and geospatial mapping, the final output is to create a gridded agriculture methane inventory at the country level in Nigeria and Colombia, which will be used as input for high-resolution satellite inversions for an updated gridded methane inventory. Last but not least, we conduct capacity building workshops focusing on agriculture methane to raise awareness among local communities.

IMEO's approach to agricultural methane is through Baseline Science Studies, which aim to develop an agriculture methane emission baseline at the country level through multi-scale measurement studies, integration with existing data and high-resolution satellite inversions. The current step is to finalize the compilation of existing data, unpublished data from government agencies and statistics, and to identify the knowledge gap that will pave the way for the design and implementation of in-situ agricultural methane measurements. Ultimately, we want to create a gridded agriculture methane inventory based on measurements from the selected countries, and to Integrate the updated inventory and measurement into high-resolution satellite inversion for more precise agricultural methane estimates at country-level.

390 Enteric methane emission factors and intensity of small ruminants production systems in Fiji – improved tier 1 approach

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Fiji has the largest population of small ruminants across the Pacific Island Countries with a total of 37,435 sheep and 143,853 goats. Small ruminant production occupies an important socioeconomic and cultural role within Fijian society, however low productivity levels raise concerns about high emissions intensity of this sector. In addition, there is no published information about the enteric emissions of small ruminants in Fiji and possible ways it could be minimized. This work aimed to utilize the Tier II framework to estimate the greenhouse gas inventory for small ruminant production in Fiji. Data on herd/flock dynamics and animal performance from 19 sheep (n=9) and goat (n=10) farms across the Northern and Western Division of Fiji were monitored for at least one full year. At the beginning of the monitoring, all small ruminants were registered and received a unique eartag ID. Participating farmers were provided a hanging scale as well as a records booklet. Farms were visited once a month by enumerators and data related to flock numbers, liveweight births, deaths, sales/household consumption/gifts recorded. Productivity parameters (e.g. liveweight gain, lambing/kidding rate and mortality rate) were all calculated based on the farm recordings. Enteric methane emission factors were indirectly calculated based on gross energy intake estimated based on animal activity data collected on farms following IPCC 2019 methodology. Liveweight gain of young animals was modelled using a quadratic linear mixed model including age, farm, species (i.e. goat or sheep), type of birth (i.e. single, twins or triplets) and sex as fixed factors and animal as a random term. Proportional productive parameters (e.g. lambing rate, kidding rate, death rate) were all calculated and compared using a generalized mixed Poisson effect model. Using the available data and literature information, total metabolic energy requirements, daily enteric methane emissions per season, and annual emissions (emission factors) for different animal classes will be calculated. Additionally, animal performance indices for goats in the country will be documented. This pioneering study in Fiji will provide the first Tier 2 enteric methane values for sheep and goats production systems, as well as estimated emission intensities. The results will contribute to improved reporting of the national livestock inventory and the development of locally appropriate mitigation strategies. Data processing and analysis are currently ongoing and will be completed in time for the GGAA 2025 conference.

421 Emission factors for enteric methane emissions from cattle in Taita County, Kenya

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As global climate change escalates, the livestock sector significantly contributes to greenhouse gas (**GHG**) emissions, particularly enteric methane (**CH₄**) from ruminants. The objective of this study was to estimate Tier 2 enteric CH₄ emission factors (**EF**) for cattle in Taita County, Kenya. The study collected activity data from 617 cattle of various categories (77 calves (<1 year), 244 young females (1-3 years), 32 young males (1-3 years), 113 adult males (> 3 years), and 119 adult females (> 3 years)). Direct measurements included live weight (**LW**), feed, and milk quality. Daily CH₄ production (**DMP**) for each cattle category was calculated using the IPCC Tier 2 methodology for three time periods: cold dry season (**CD**), short rainy and hot dry (**SR+HD**), and long rainy season (**LR**). The annual Tier 2 EF was estimated as the weighted average of the DMPs in the three time periods and compared with the IPCC (2019) Tier 1 default EFs. The average feed digestibility was estimated from diet composition at 54.5%, 55.9%, and 52.3% during the CD, SR+HD, and LR seasons, respectively. The DMPs differed among seasons, with the highest DMP recorded in the SR for all categories except young males, which had its peak in the LR season. The highest EF was recorded for adult females at 85 kg CH₄/head/year, followed by adult males (73 kg CH₄/head/year), young males (68 kg CH₄/head/year), young females (61 kg CH₄/head/year), and calves (32 kg CH₄/head/year). Compared to Tier 1 EF, it was lower for adult females (74 kg CH₄/head/year) and similar for calves (31 kg CH₄/head/year). In most categories, except adult males, the Tier 1 default values were lower than the Tier 2 estimates largely due to the lower LW: in young males (204 vs 236 kg) and females (204 vs 216) and lower daily milk yield in adult females (2.4 vs 5.6 kg/day). The discrepancies between Tier 1 and Tier 2 estimates emphasize the importance of using local data to inform emission factors, as the IPCC default values for Africa may not accurately reflect the unique conditions present in a region.

423 Simulated seasonal methane emission factors for sheep in the highveld of South Africa

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Accurate estimations of livestock greenhouse gas (**GHG**) emission factors are of critical importance when reporting inventories, identifying key emission sources, developing mitigation strategies and informing policy. Improving direct livestock emission factors using country specific emissions would support accurate GHG inventories.

The objective of the study was to develop enteric emission factors from sheep fed diets representative of commercial extensive production systems in the highveld of South Africa. The experiment was run as a completely randomised design with four treatment diets and four replicates per treatment using Merino rams of 55.6 ± 3.5 kg. The treatments represented a typical rangeland quality and consisted of an *Eragrostis curvula* and *Digitaria eriantha* hay mixture in the South African highveld with a summer rangeland (CP: 8.5%), an autumn rangeland (CP: 6.9%) and winter rangeland (CP: 4.3%). All rangeland diets received commercial supplements as described by industry standards. A phosphate supplement for the summer rangeland, a condition supplement for the autumn rangeland, and a lamb and ewe supplement for the winter rangeland. A fourth diet consisting of a ram, lamb and ewe total mixed ration (**TMR**) which is an acceptable standard when rangeland is limiting in the region was included in the trial. The trial consisted of a 14-day adaptation period, a 7-day total tract digestibility trial and a 4-day enteric methane emission study using closed circuit respiration chambers.

The total dry matter digestibilities from the dietary treatments ranged from 65.8 to 51.8% with the TMR representing the highest digestibility. Total enteric methane emissions were the highest for the total mixed ration (25 g/d) followed by the summer rangeland diet (23.2 g/d) and then decreased to the winter rangeland diet (17.4 g/d). Methane emission intensity (g/kg DMI) was the lowest for the TMR compared to the summer rangeland and phosphate supplement diet with 16.0 compared to 23.5 g/kg DMI respectively. The data of the current study was comparable to Tier 2 emission factors estimated for commercial Merino sheep developed for South Africa of 20.7 g/kg DMI.

The development of country specific methane emission factors can increase the accuracy of national livestock GHG emissions inventories. The current study aided in the improvement of seasonal activity data and experimental emission factors for Merino type sheep validating previously estimated IPCC Tier 2 emission factors for small stock in the highveld of South Africa. The collected data has been contributed towards the development of a country-specific methane emissions yield model for South Africa.

431 Enteric methane emission factors for cattle in rangeland systems: a case study in Kenya

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Livestock production in Kenya contributes significantly to greenhouse gas emissions, particularly through enteric fermentation and methane (CH₄) emissions. The objective of this study was to estimate Tier 1 and 2 enteric CH₄ emission factors (EFs) for rangeland cattle production. Using standardized Intergovernmental Panel on Climate Change (IPCC) Tier 1 and 2 methods, enteric CH₄ EFs (kg CH₄/head/year) were calculated based on animal energy requirements derived from activity data. This included animal characteristics, performance, feed composition, and diet gross energy to estimate feed digestibility. Data were collected from 1,343 cattle across three locations in Kenya's pastoral landscapes: Kapiti Research Station and Wildlife Conservancy (719 cattle), Olkiramatian Community Ranch (299 cattle), and Shompole Community Ranch (325 cattle). Kapiti, owned by the International Livestock Research Institute (ILRI), is an extensive ranching system where livestock graze in grasslands designated for paddocks throughout the day. In contrast, Olkiramatian and Shompole ranches, owned by pastoralists, feature designated settlement and farming areas with free-ranging animals during the day that are kept in enclosures overnight. The cattle at Kapiti included pure Boran breeds and Holstein-Boran crossbreeds. Shompole ranch kept crossbreeds (Boran with zebu and Boran with Sahiwal), while Olkiramatian maintained both crossbreeds (Boran with zebu and Boran with Sahiwal) and dual-purpose indigenous cattle. Activity data were collected for four seasons during one year for Kapiti Ranch and two seasons in Shompole and Olkitamarian. Emission factors for the cattle category (adult females (≥ 3 years), adult males (≥ 3 years), young males (1-3 years), young females (1-3 years), and calves (< 1 year)) were estimated for wet and dry seasons, allowing the calculation of mean annual EFs (+/- SEM). The annual EFs (mean ± SE) for adult females varied significantly among the three locations: Kapiti had the highest enteric CH₄ EF (90 ± 1.4 kg CH₄/head/year), followed by Olkiramatian (66 ± 1.5 kg CH₄/head/year) and Shompole (54 ± 1.2 kg CH₄/head/year). Kapiti had the highest EF for the other cattle categories, whereas there was no difference between EFs for Olkiramatian and Shompole. There was a significant difference in the mean annual EF of all cattle categories: Kapiti (64 ± 0.9 kg CH₄/head/year), followed by Olkiramatian (52 ± 1.2 kg CH₄/head/year), and Shompole (42 ± 1.0 kg CH₄/head/year). A comparison of the estimated Tier 2 EFs with Tier 1 values revealed that Kapiti exhibited mean EFs 2 - 26 % higher than the IPCC Tier 1 default values. At the same time, EFs for Olkiramatian (7-19 %) and Shompole (5-41%) were lower. These differences correspond with variations in live weights across the ranches, as heavier animals have higher energy requirements, contributing more to emissions if sufficient feed is provided. For example, the comparison of Tier 2 and Tier 1 live weights are as follows: Kapiti (374 vs. 356 kg), Olkiramatian (251 vs. 356 kg), and Shompole (259 vs. 356 kg). The findings highlight significant variability in methane emission factors across rangeland systems within pastoral setups, emphasizing location-specific data's importance in refining national greenhouse gas inventories and identifying feasible mitigation strategies.

434 Enteric methane emission factors for pastoral cattle: A case study in Ethiopia's Yabello district

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The objective of this study was to estimate enteric methane (**CH₄**) emissions of cattle in pastoral systems in the Yabello district of Ethiopia using the Intergovernmental Panel on Climate Change (**IPCC**) Tier 2 approach. A longitudinal study was undertaken in all four seasons (short rain, short dry, long rain and long dry) to obtain activity data required to estimate emission factors (**EF**, kg CH₄/head/year) for different cattle categories (adult females (≥ 3 years), adult males (≥ 3 years), young males (1-3 years), young females (1-3 years), and calves (< 1 year)) using the Tier 1 and 2 methods. The EF was calculated by animal category for each season. By averaging the estimated enteric CH₄ emissions across these seasons, an annual EF for the cattle was derived. Mean enteric EFs ranged from 23 kg CH₄/head/year in calves to 68 kg CH₄/head/year in adult females. The flock emitted on average 56 kg CH₄/head/year. For all cattle categories, the EF was lower than the IPCC Tier 1 African default values primarily due to the lower Tier 2 live weights (**LW**). For instance, adult females had lower Tier 2 LW than Tier 1 LW (307 kg vs 356 kg). The Tier 2 daily CH₄ production (**DMP**) showed differences across seasons, with the short rainy season exhibiting the highest DMP in adult animals (207 kg CH₄/day) and calves (83 kg CH₄/day), while the long rainy season showed higher DMP in young males (127 kg CH₄/day) and females (118 kg CH₄/day). This variation corresponded with seasonal variations in LW, LW flux, and milk yield, which in turn affect intake levels. This highlighted the importance of considering regional activity data to estimate emissions factors and specific livestock categories when assessing CH₄ emissions. The research emphasized the necessity of seasonal assessments to capture the variability in enteric CH₄ production effectively.

442 Methane emission factors from enteric fermentation in Beninese cattle breeds

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Livestock sector contributes significantly to greenhouse gas (**GHG**) emissions through methane (**CH₄**) from enteric fermentation. To direct efforts on mitigating climate change, accurate estimation of GHG emissions from livestock production is needed. The objective of this study was to estimate enteric methane (CH₄) Emission Factors (**EFs**) for the Azawak and Borgou cattle breeds according to the 2019 refinement of the 2006 Intergovernmental Panel on Climate Change (**IPCC**) using the Tier 2 approach. Using animal characteristics and performance data collected from 372 cattle from Okpara Breeding Farm, net energy requirements were estimated. Five animal groups were considered according to age: milking cows, bulls, Heifers, steers and calves. We found that the average emission factors for Azawak were 78.3, 84.5, 51, 55.6 and 37 kg CH₄/head/year for cows, bulls, heifers, steers and calves respectively. For Borgou, they were 68.5, 58.8, 48, 52.3 and 51 kg CH₄/head/year for cows, bulls, heifers, steers and calves respectively. Globally, the mean emission factor for Azawak and Borgou cattle breeds were 62.6 and 54.1 kg CH₄/head/year respectively. The findings from this study can serve as a baseline for the national emission inventory in Benin.

472 Development of a standardised, science-based method for measuring farm-level greenhouse gas emissions

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Global customers and regulators are progressively demanding that New Zealand's exporters (and therefore our farmers) report and reduce their GHG emissions. This project overview highlights the work undertaken by New Zealand's Ministry for Primary Industries, together with domestic agricultural sector organisations, to met this demand through the development of a standardised, science-based methodology for estimating farm-level biogenic methane and nitrous oxide greenhouse gas emissions. Having a transparent and consistent approach to how emissions are calculated is also a first step to ensuring farmers have access to tools they can trust and making it easier for them to measure and mitigate on-farm emissions.

This methodology, underpinned by New Zealand's agricultural inventory model, is an accessible, low-cost, and auditable design that uses farm-specific data readily available to most farmers. This includes livestock details (gender, age, stock class), supplementary feed, production including milk and liveweight gain, fertiliser use, and farm location. A Technical Advisory Group supported the development of the standardised methodology and its scientific validity.

To ensure transparency, the Ministry has also publicly released the codebase associated with the methodology. It is anticipated that there will be annual releases to reflect the release and adoption of new technologies and scientific evidence and that any substantive changes to the methodology will undergo assessment by a panel of science and sector nominees prior to adoption.

In the next phase of this project, the Ministry is working with agricultural and science experts to standardise and rationalise the number of farmer-facing emissions calculators. Prior to this project there were more than 10 calculators available offering a range of methods to estimate emissions causing confusion and adding to the administrative burden for farmers and processors.

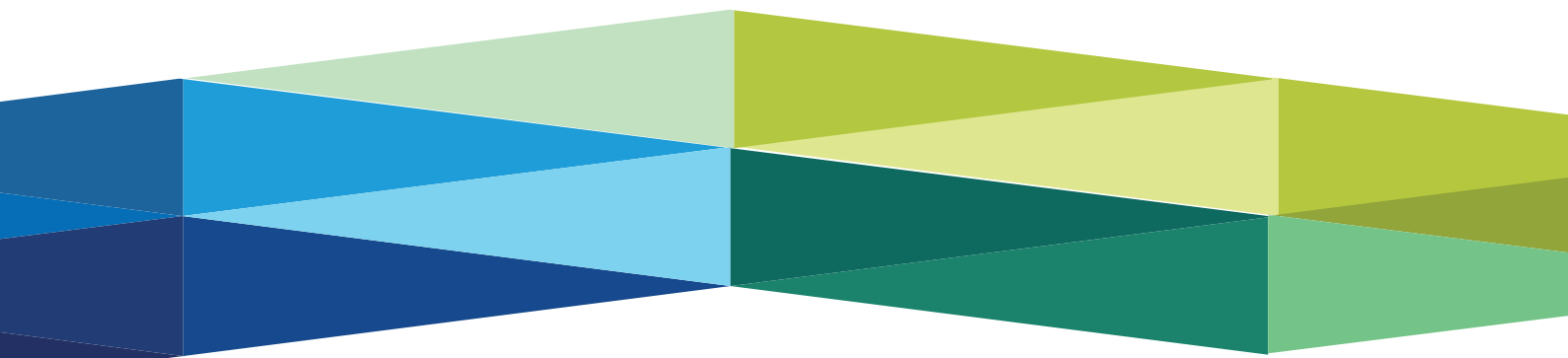
National greenhouse gas inventories remain a critical tool linking policy with mitigation efforts and help countries and agricultural sectors to model greenhouse gas emissions and target areas to focus mitigation efforts. The project complements New Zealand's investments to improve its national GHG inventory to ensure the outputs reflect our unique pastoral farming systems and climate. This expertise is central to New Zealand's international outreach programme providing practical assistance and training to help countries move from Tier 1 to Tier 2 inventories.



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Theme 9: Measurement techniques for greenhouse gas emissions



134 Cattle welfare in Ghana: linking stress management to health, productivity, and climate resilience

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Enhancing cattle welfare is vital for sustainable livestock systems under growing climate pressures in sub-Saharan Africa. This study investigated cattle welfare practices in Ghana across farming, transport, and slaughter stages and assessed implications for animal stress, meat quality, and climate resilience. A cross-sectional survey was conducted in seven regions using convenience sampling, involving 318 farmers, 78 transporters, and 450 butchers. Data collection combined structured questionnaires and direct observations. Descriptive statistics and ANOVA were used to analyze stress indicators across regions and transport durations. Quantitative welfare metrics included blood cortisol levels, measured via ELISA from samples collected immediately post-slaughter; meat pH assessed 45 minutes after slaughter using portable pH meters; and carcass bruising rates recorded via visual inspection. Results showed 95% of farmers prioritized basic needs (hunger/thirst), but none addressed behavioral needs. Transport stressors – prolonged transit (average: 18 hours), overcrowding, and poor vehicle design – contributed to bruising in 53% of carcasses. In slaughterhouses, 64% of cattle exhibited signs of severe stress, and 36% showed aggressive behavior due to rough handling. Elevated cortisol (mean: 43.4 ng/mL) and pH levels (mean: 6.5) were significantly associated with longer transport durations ($p < 0.05$), resulting in high rates of Dark Firm Dry (**DFD**) meat, reducing market value and consumer acceptability. These stress-related inefficiencies compromise productivity and resilience, particularly under climate-driven challenges like feed scarcity and heat stress. Proposed interventions include community-led animal welfare networks for pooled veterinary and transport resources; rotational grazing systems integrating indigenous knowledge to reduce drought vulnerability; retrofitting transport vehicles with local materials to reduce injury; and mobile welfare training units for on-site education. By strengthening welfare along the value chain, Ghana can enhance livestock adaptability, reduce economic losses, and build climate-resilient production systems.

161 Real-time, online monitoring of ammonia and GHG emissions in livestock with Cavity Ring-Down Spectroscopy

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Agricultural activities and processes emit greenhouse gases like CO₂, CH₄ and N₂O, as well as NH₃. While greenhouse gas emissions affect the global climate, ammonia is a toxic pollutant that affects human health and the environment on a regional level, e.g., by threatening biodiversity. To reduce the impact of livestock production on the environment, there is a high need to quantify GHG as well as ammonia emissions from barns and to develop mitigation options. Accurate ammonia measurements at the ppb to ppm level are challenging due to the high reactivity of ammonia and its tendency to adsorb to surfaces making it one of the most difficult trace gases to measure.

Here, we will present the new G2509 Cavity Ring-Down Spectroscopy (**CRDS**) analyzer that is optimized for continuous measurement of ammonia, while also reporting the greenhouse gas concentrations of CO₂, CH₄ and N₂O. The measurement response time of NH₃ is sped up with low-reactivity internal materials and a high flow rate. The CH₄ concentration range of the analyzer is extended to 800ppm to account for large variations in CH₄ concentrations in barns. The G2509 analyzer adapts the excellence of the G2508 greenhouse gas analyzer that is commonly used to study greenhouse gas fluxes from soils by implementing key design elements of the PI2103 analyzer that is dedicated to low level ammonia monitoring at air quality stations. Here, we will compare key features of the new multi species G2509 analyzer and the single species ammonia PI2103 analyzer and discuss best practices to achieve the highest level of data quality for continuous ammonia measurements.

234 Use of the portable Laser Methane Detector® in livestock – 15 years on

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Since the first feasibility study on use of the hand-held laser methane detector (**LMD**) in ruminants in 2009 different research groups have adopted the technique. The evolution has been around testing the robustness, accuracy and precision of the data, comparison with traditional methane measurement methods, application in different environments and species, and development of measurement protocols. The importance of quantification and monitoring of enteric methane emissions from livestock cannot be over emphasised and hence taking stock of the viability and applicability of any promising proxies is vital. The current paper aimed to investigate the LMD's contribution and significance to current state of knowledge towards enteric methane emissions mitigation in the last 15 years.

To understand the potential consequences of the introduction of the LMD in livestock, the study used impact analysis approach. Scientific papers based on original research that have been published in the past 15 years, were used as the study subjects. Review papers and ancillary papers from the pioneering authors were excluded from the study.

In the period between the year 2011 and 2024, there has been a total of 21 peer reviewed publication translating into an average of 1.40 papers published on the use of the LMD every year in the past 15 years. As might have been expected, the majority of the papers that have been published so far have been on dairy cattle (n = 11). Only two papers have been on beef cattle. The other species have been sheep (n = 3), goats (n = 2) and Mediterranean buffaloes (n = 2). The predominant production system is the intensive system whilst only two of the papers were published in extensive livestock production systems. Geographically, the studies have been from the United Kingdom, Germany, Denmark, Poland, Switzerland, France, Portugal, Republic of Korea, Japan, South Africa, Ethiopia, Malawi, Italy, and Spain. Results obtained in these papers have demonstrated that the LMD is able to discriminate animals based on diet, breed, lactation stage, season, and health status. The association and relationship with existing methods such as the metabolic chamber and another proxy, GreenFeed have shown positive and significant correlations ranging from 0.57 to 0.77. Further, phenotypes generated from the LMD data have been used to characterise the underlying genetic parameters for enteric methane emission in ruminants. Of the 21 studies, 20 found the LMD a very promising tool with potential to be used in ruminant studies. One study reported the need to refine the technique to enable prediction of daily CH₄ emissions. Since it was published, the first paper has received 118 citations.

With the advantage of being specific, non-invasive, rapid, cost-effective, and portable, the use of LMD in livestock has increased. Work from different researchers as shown strong correlation and agreement with metabolic chambers and GreenFeed. Being a handheld equipment, the LMD requires more labour than automated equipment. Although providing meaningful data, future considerations should be on technique development, recording protocols, and data handling.

255 A field-deployable device for continuous methane and carbon dioxide emission measurement in cattle.

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Zero Emissions Livestock Project (**ZELP**) is transforming sustainable livestock management with an innovative device designed to measure methane (**CH₄**) and carbon dioxide (**CO₂**) emissions from cattle, called Sense. This technology enables continuous, non-invasive monitoring in natural environments, providing more precise greenhouse gas (**GHG**) emission data and addressing the constraints of traditional respiration chambers. Methane emissions from cattle significantly contribute to greenhouse gas accumulation, highlighting the need for effective monitoring systems to advance environmental sustainability. Respiration chambers are considered the gold standard in the field of methane measurements and, while accurate, are costly, labour-intensive, and restrict natural behaviour.

The objective of this work was to test the accuracy of Sense when compared to the respiration chambers and determine the feasibility of using it as a device to accurately measure CH₄ and CO₂ from cattle. Sense integrates advanced sensors, including near infrared optical and catalytic gas sensors, thermistors, thermocouples, accelerometers, and inertial measurement units (**IMUs**), all of which are used to capture the exhaled gases. These components are mounted on the animal's face using a ventilated neoprene face sock, designed to prevent abrasions and allow airflow, avoiding sweat or dirt buildup. To validate the accuracy of Sense, three dry-heifers, fitted with the Sense device, were tested in respiration chambers over a 3-week period, with two separate 3-day testing sessions conducted, separated by a one-week break. Sense continuously captured CH₄ and CO₂ concentrations and data transmission was conducted via onboard storage and wireless connectivity.

Methane emissions were measured at 15-minute intervals, and the device's performance was compared to chamber data, for which the correlation (*r*) and RMSE were calculated by means of machine learning (**ML**) models. The trial also assessed thermistor accuracy, prototype durability, and animal welfare through behavioural observations and respiration rate (**RR**) checks. Results show the device provides comparable emission readings to respiration chambers. Average correlations observed between Sense and the respiration chambers in the two testing periods demonstrated a positive correlation of *r* = 0.6 and an RMSE <20% for the CH₄ measurements. Similarly, when looking at CO₂ measurements, a correlation of *r*=0.4 and RMSE of <17% was observed. The *r*-value for CO₂ was slightly lower due to less variability in CO₂ measurements throughout the testing period.

When looking at the device's impact on animal behaviour, RR remained within the normal range of 26–50 breaths per minute, and behavioural observations showed no signs of distress, indicating how natural behaviours were maintained throughout the duration of the testing period. The results show promise on the ability that Sense has to address the limitations of traditional respiration chambers by enabling accurate, real-time monitoring of methane and carbon dioxide emissions in natural environments. No changes in animal behaviour were observed, highlighting the device's potential for long-term deployment without compromising animal welfare.

Further work is required, which will include further barn and grazing deployments, and additional benchmarking versus existing measurement techniques, to further validate the Sense device.

265 Sniffers: intake sampling location significantly influenced observed concentration, between animal variability, and ranking accuracy

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Estimating enteric methane (**CH₄**) emissions from large animal populations require accurate and cost-effective measurement techniques. The sniffer technique used an air intake and gas concentration sensors to continuously measure local gas concentrations (airflow 1L/min). Sniffers are usually placed near a milking robot (**AMS**) with the intake mounted near the animal's muzzle in the AMS feed trough. The objective of this project was to compare the variability of measurements and ranking coefficients between sniffers arranged closely on the AMS to the GreenFeed system (**GF**). It was hypothesized that the location of sniffer intake will affect its response and accuracy. Ten complete sniffer systems (SimpleScan; C-Lock Inc.) were used. Sample intakes for 9 units were arranged in a 3x3 grid, spaced 15 cm apart in the AMS trough and 1 unit was mounted directly above the AMS. Each sniffer simultaneously recorded CH₄ ppm on a 1-sec basis for further processing to calculate average CH₄ ppm for each animal visit. A GF was located inside the free-stall pen to simultaneously measure gas fluxes from the same animals. Repeated measures of CH₄ production (g/day) from GF and sniffer CH₄ concentrations (ppm) were collected for 6 months from 95 lactating dairy cows, resulting in 173,736 sniffer (19,304 per sniffer) and 9,247 GF records. If a cow visited GF or sniffer fewer than 8 times in a week, data for that cow during that week were removed. Methane measurements were averaged weekly, resulting in a final data set of 4,230 records at the cow-week level. Repeatability was calculated as the proportion of the total variance explained by random between-animal variance. Variance components were extracted by fitting linear mixed effect models, where GF and sniffer measurements were response variables. Animal breed, parity, and lactation stages were fixed effects, and animal was included as a random effect. Concentration responses from different sniffer intake locations varied around the overall mean $\pm$ SD (339 ± 169.6 ppm), ranging 232 to 574 ppm across sniffer locations. Sniffer 5 (the center) recorded the greatest concentration (526 ± 165.9 ppm), while Sniffer 6, immediately next to Sniffer 5, averaged 234 ± 77.6 ppm, approximately 2.25-fold less. Small differences in sniffer locations resulted in significant measurement variation. The between-cow coefficient of variation was greater for sniffers compared to the GF (31% vs. 15%). The average repeatability of sniffers was lower than the repeatability of GF (0.49 vs. 0.66). Sniffer 5 had the greatest repeatability (0.61) and sniffer 8 the lowest (0.14). Moderate Spearman's correlations were observed between sniffer concentrations and GF fluxes with sniffer 5 greatest ($r = 0.50$, $P < 0.001$) and the others ranged 0.30 to 0.38 ($P < 0.001$). The placement of sniffer intakes significantly affected CH₄ concentration measurements and their correlation with fluxes measured using GF. Placing the sniffer in the center of AMS provided the most agreement with GF. Analyses will further evaluate the agreement between sniffer (concentration) and GF (fluxes) accounting for different sources of variation when ranking cows by high and low CH₄ emitters.

333 Evaluating the Sniffer method for methane emission in dairy cows differing in feed efficiency

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Selecting low methane (**CH₄**) emitting but feed-efficient cows is crucial for sustainable dairy production. One approach to assessing feed efficiency in dairy systems is residual feed intake (**RFI**), where lower RFI values indicate greater feed efficiency. A cost-effective method for estimating CH₄ emissions is the Sniffer method. The Sniffer method estimates CH₄ by multiplying measured CH₄:CO₂ ratio by predicted CO₂ production, which is calculated based on metabolic body weight and energy-corrected milk yield. However, this method may cause bias in methane estimation based on CO₂ production and result in the selection of inefficient cows. This study investigated the relationship between CH₄ measured in respiration chambers, CH₄ estimated by the Sniffer method, and feed efficiency as determined by RFI. A dataset of 1,060 individual records from 37 European respiration chamber studies was used for the analysis. In the present study, CH₄ (g/day) and CO₂ (g/day) data from the respiration chambers were used to calculate their ratio. Residual feed intake was calculated as the difference between the observed dry matter intake and dry matter intake predicted based on metabolic body weight, energy-corrected milk, and retained energy, which was calculated from the part of gross energy intake used for heat production, CH₄ energy, milk energy, fecal energy, and urinary energy excretions. Regression analyses showed that CO₂ production was overpredicted when using the equation in the low RFI group of efficient cows (mean predicted: 12.2 vs. observed: 11.6 kg/day) and underpredicted in the high RFI group of inefficient cows (mean predicted: 12.3 vs. observed: 13.4 kg/day), with R² values of 0.71 and 0.70, respectively ($P < 0.01$). This resulted in the overprediction of CH₄ in the low RFI group of efficient cows (mean predicted: 380 vs. observed: 362 g/day) and underprediction in the high RFI group of inefficient cows (mean predicted: 384 vs. observed: 419 g/day), with R² values of 0.78 and 0.79 and adjusted RMSE of 43.1 and 51.5, respectively. In conclusion, our data suggest that the Sniffer method overestimates CO₂ production and thus CH₄ emissions in efficient cows and underestimates them in inefficient cows, leading to the risk of selecting inefficient animals. This underscores the need to improve CH₄ and CO₂ prediction methods to ensure more accurate selection of low-emitting, feed-efficient cows.

337 Measuring enteric methane in cattle using portable accumulation chambers

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Selective breeding has been shown to be viable for reducing methane (**CH₄**) emissions in ruminant livestock. It relies, however, on measuring CH₄ in farmed livestock which is particularly challenging for large animals such as cattle. Several measurement technologies (i.e., GreenFeed, sniffers, respiration chambers) are available for cattle but present significant challenges for affordable large-scale application in extensive, pastoral based systems. An alternative technology to obtain short-term commercial CH₄ measurements on-farm that has been successfully used in sheep and goats is the portable accumulation chamber (**PAC**). To our knowledge, portable chambers have never been tested in cattle. In this project, we designed and engineered PAC units specifically for cattle. Six of these units were subsequently used to measure CH₄ and carbon dioxide emissions from 125 Angus-Hereford (pure or crossbred) heifers across two-time points that were 2 weeks apart. The heifers were from a commercial enterprise, ~10 months of age, grazed at pasture under standard New Zealand farming conditions, and weighed an average of 250kg. These PAC units were shown to provide gas measurements in line with expected values when compared to sheep, and no animal behaviour issues were observed during the trial. The repeatability of the gas traits from PAC were estimated to be 0.35 ± 0.08 for CH₄ emissions (g/day), 0.13 ± 0.09 for carbon dioxide (**CO₂**) emissions (g/day), and 0.31 ± 0.09 for the ratio trait of moles of CH₄ relative to the sum of moles of CH₄ and moles of CO₂ ($\text{CH}_4/(\text{CH}_4 + \text{CO}_2)$). These results suggest that the newly designed cattle PAC units are a suitable high-throughput method for the measurement of CH₄ emissions from young beef cattle. These units would enable large-scale on-farm phenotyping for the purpose of ranking animals on their CH₄ emissions, which in turn would facilitate the development of breeding schemes to select for low CH₄ emitting cattle. Next steps are to obtain sufficient number of measures to estimate heritabilities and variance components in CH₄ traits obtained from PAC for cattle.

389 Measurement of enteric greenhouse gas from buffaloes: Comparison of the GreenFeed and Sniffer system

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Different techniques according to the invasiveness on the animal, the accuracy of the records and the cost of the instruments, are available to perform on-farm measurement of enteric emissions of greenhouse gas (**GHG**). Presently, the most reliable system to quantify repeated short-term breath measurements of individual enteric methane (**CH₄**) and carbon dioxide (**CO₂**) emissions under farm conditions is the GreenFeed (**GF**). On the other hand, the sniffer system (**SN**), made by a nose placed at the feed bunk of a milking robot, is a less expensive system that also allows more animals to be monitored. To the best of our knowledge, none of these systems have been used before in buffaloes.

The study aimed to quantify the enteric CH₄ and CO₂ emissions on Mediterranean dairy buffaloes using a GF unit (C-Lock Inc., USA) and a sniffer system (Tecnosens, IT). The trial was conducted during the summer months of July and August, lasted 30 days and engaged 20 animals. Ten lactating buffaloes were free to visit a milking robot equipped with the SN, which permitted continuous measurement and recording of CH₄ and CO₂ concentrations (ppm); in an adjacent pen, ten ear-tagged buffaloes milked through a conventional milking parlour were monitored by a GF unit. A training period of 4 weeks was needed to make animals voluntarily approaching the GF unit. SN and GF groups were balanced according to the days in milk (201 ± 143 and 218 ± 166) and parity (1.3 ± 0.7 and 1.7 ± 0.8). Both groups fed the same total mixed ration and the same concentrate was supplied by the milking robot and GF unit.

Sniffer raw data were processed to clean the dataset from outliers, negative values and background gas concentrations. Once SN data were standardized, the individual daily CH₄ production (g/head/d) was estimated considering the amount of CO₂ predicted from animal traits and the ratio of CH₄ and CO₂ measured by SN. In the GF group, individual daily CH₄ and CO₂ production (g/head/d) were provided by the system.

The average production of CH₄ and CO₂ measured by the GF was 333.5 ± 67.3 g/day and $9,535.9 \pm 1,352.7$ g/day, respectively; the estimated emissions for the SN group were 269.8 ± 31.8 g CH₄/day and $9,533.1 \pm 649.7$ g CO₂/day. This first use of GF in Mediterranean dairy buffaloes made available a first benchmark of the emissive levels for this genotype. More research is required to refine the model for calculating emissions by SN technology, and validate the SN method for buffalo production systems. The spread of the milking robot in dairy buffalo farms will enable a rise in the number of animals monitorable by SN that will help develop a more accurate calculation of enteric methane emission factors.

430 Analysing the suitability of small animal GreenFeed systems for measuring methane emissions from young calves

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The negative impacts of livestock farming to global warming has become a great concern worldwide, primarily from enteric fermentation, which significantly contributes to the global greenhouse gas inventory. It is crucial to accurately quantify methane (**CH₄**) emissions to develop effective mitigation strategies. In recent years, the GreenFeed system (C-Lock Inc.) has emerged as a promising technology for measuring CH₄ emissions from ruminants. The GreenFeed system encourages animals to visit the feeding station that captures and analyses burps to quantify CH₄ emissions. The present study evaluated the suitability of the small-animal GreenFeed system for estimating methane emissions in 20 calves, comprising Aberdeen Angus and British Blue Cross breeds with an average age of three months at the start of the trial. Data were collected daily over a 13-week period, spanning from December 2023 to March 2024. Further, the processed data was collected from the Greenfeed data portal managed by C-Lock Inc., which recorded the CH₄ emission data (CH₄ in g/d) from each animal. Our analysis focused on primary aspects like, average number of visits, duration and total methane output per animal, reflecting the efficiency of using small animal GreenFeed for the young calves. The results from the study showed an average of 270 visits per animal during the monitoring period, with a range between 201 (minimum) and 351 (maximum) visits per calf during the study period. On average, calves visited the GreenFeed system 4 times per day ($\sigma = 1.14$). When looking at the average visit duration per animal, it is found that mean visit duration was 3.88 ± 1.00 minutes, with minimal variation across individuals, indicating consistent behavioural engagement. Hence, it is evident that the GreenFeed system can efficiently capture CH₄ emissions within the time frame, which is ideal for young calves. This slight variation suggests that the individual behaviour of an animal may affect the time spent at the system, but overall, the engagement times are consistent. The average CH₄ emission across all events was 94.08 ± 30.55 g/day, indicating moderate variability in the emissions data. Although the reason for variations in methane emissions among individual calves were not the primary focus of this study, which instead aimed to evaluate the suitability of the small-animal GreenFeed system for use in young calves. Hence, the observed variability warrants further investigation, and future studies will consider potential contributing factors such as breed, diet, body weight, and age of the calves. Overall, the consistency in visit frequency supports the reliability of the small-animal GreenFeed system for estimating methane emissions in young calves. The findings provide an insight into small GreenFeed's efficacy for studying CH₄ emissions in young calves, supporting the potential for its application in various research on early interventions on methane mitigation.

449 Validation and reliability of methods to measure enteric methane emissions in dairy cattle

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Accurate measurement of enteric methane (**CH₄**) emissions is crucial for assessing mitigation strategies and their environmental impact. Respiration chambers, considered the gold standard due to their precision, are limited by high costs, low throughput, and potential impact on animal behavior, prompting the adoption of alternative methods such as headbox systems. Validating these methods is essential to ensure reliable CH₄ quantification across varying emission levels. Two studies conducted at the Large Animal Research and Teaching Unit at Cornell University (Ithaca, NY, USA) evaluated the GreenFeed system by comparing individual animal spot CH₄ measurements with those obtained from open-circuit respiration chambers. Study 1 involved 8 pregnant Holstein heifers (18 ± 0.49 months of age, 164 ± 4.42 days of pregnancy, and 556 ± 39.4 kg of body weight), while study 2 included 12 late-lactation pregnant Holstein cows (202 ± 12 days in milk, 46 ± 6.53 kg of milk/day, and 710 ± 29.8 kg of body weight). Prior to data collection, animals were trained to use both systems. In study 1, heifers completed five 6-day repetitions, with CH₄ measured for 3 days using the GreenFeed system and 3 days using respiration chambers. In study 2, cows completed three 9-day repetitions, with CH₄ measured for 3 days each using the GreenFeed system, respiration chambers, and an additional method (data not included). The GreenFeed unit remained stationary, and measurements ($n = 8$) were taken every 9 hours to ensure representative 24-hour sampling. Environmental conditions in the facility were controlled ($16\text{--}20^\circ\text{C}$ and 30 and 57% relative humidity). Animals were fed a total mixed ration, with feed and water provided ad libitum. Body weights were recorded at the start and end of the experiments, and dry matter intake was measured daily throughout the experiments. Data were analyzed separately for each study using PROC MIXED of SAS, and correlation coefficients were calculated in R. Dry matter intake did not differ across methods ($P > 0.05$) for heifers (mean = 8.72 kg/day) or cows (mean = 25.1 kg/day). As expected, CH₄ emissions were higher ($P < 0.01$) when measured in chambers compared to GreenFeed, with cows emitting 546 vs. 402 g/day and heifers emitting 220 vs. 189 g/day, respectively. Emissions of carbon dioxide were also higher ($P < 0.01$) when measured in respiration chambers, both for cows (15.6 vs. 13.3 kg/day) and heifers (8.64 vs. 8.38 kg/day). Similarly, CH₄ yield was greater ($P < 0.01$) in respiration chambers for heifers (25.1 vs. 22.1 g of CH₄/kg of dry matter intake) and cows (22.8 vs. 16.5 g of CH₄/kg of dry matter intake). As observed in mature, lactating cows, the higher CH₄ yield corresponded with a greater difference between the two methods. Lin's concordance correlation coefficient indicated poor agreement between the systems for CH₄ emissions in heifers (0.06 [-0.06, 0.17 95% CI]) and cows (0.09 [0.006, 0.174 95% CI]). Overall, our findings suggest that CH₄ emissions measured using respiration chambers and the GreenFeed system show insufficient agreement to be considered interchangeable, indicating the need for calibration factors or methodological adjustments.

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A photograph of an elderly man with a grey beard and a large red turban, looking towards the camera. He is holding a wooden stick. In the background, a herd of white sheep is grazing in a dry, dusty field with some trees and a small building in the distance.The ILRI and CGIAR logos are displayed in the bottom left corner of the image.

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